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# Soybean Genetics Newsletter



**Volume 6**

**April 1979**

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Department of Agronomy  
and Department of Genetics  
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## I. FOREWORD

The Research Notes in Volume 6 of the Soybean Genetics Newsletter reflect the international flavor of the studies directed to improving the quantity and quality of soybeans. Various methods of achieving and/or discovering mutants, and analyzing their possibilities, are being studied all over the world. Ten countries, from five different continents, are represented in this volume of the Soybean Genetics Newsletter. We are deeply appreciative of the continuing interest and enthusiastic support of soybean researchers all over the world.

Workers whose efforts made this volume possible were Carol Winger, David Stelly, Joan Oesper, Pat Muir, Holly Heer and Ann Clark. I gratefully acknowledge their assistance.

The United States Department of Agriculture continues to support the Soybean Genetics Newsletter, enabling us to mail it to interested scientists, upon request, without charge.

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## II. ANNOUNCEMENT

The second volume of the Soybean Rust Newsletter has been published by International Working Group on Soybean Rust on January, 1979. Single copies of the newsletter can be obtained free by writing to:

Mr. S. Shanmugasundaram  
Secretary, IWGSR  
AVRDC, P.O. Box 42  
Shanhua, Tainan 741, Taiwan  
Republic of China

### Request for contributions to the third issue of "Soybean Rust Newsletter"

Research articles, reports, notes, announcement of resistant or tolerant germplasm, and any other news item related to soybean rust are requested, and they will be accepted until November 1979. Address all correspondence regarding the SRN to the above address.

### Rules for contributions

- 1) Information in the SRN will be informal to stimulate the exchange of ideas and information among soybean rust scientists. SRN articles may be preliminary in nature and speculative in content, and should not be regarded as equivalent to papers published in formal scientific journals. Even so, such reports can be very valuable and helpful, if viewed in the proper perspective. Data presented in the SRN are not to be used in other publications without the consent of the respective authors.
- 2) Contributions should be in English, typed double spaced on 8½" by 11" pages. You may send as many separate contributions as you wish. Send two copies for each article.
- 3) Correspondence regarding an article should be on a separate page.
- 4) Photographs should be glossy black/white prints of high quality with good dark and light contrasts. Drawings for graphs and charts should be prepared with India ink on good quality tracing paper. Typewritten matter is not usually acceptable on graphs and charts. A good size for photographs is 5" by 7" and drawings is what will fit on an 8½" by 11" page.
- 5) Except for possible minor editing, manuscripts will be published as received from contributors.
- 6) Title your report, place your name(s), name of university, institution or company under the title. Please give complete address. [For contributors outside Taiwan (R.O.C.), please send reports by airmail.]
- 7) Citations of recent publications on soybean rust are specifically solicited.

### III. REPORT OF SOYBEAN GENETICS COMMITTEE

A) The current members of this committee and the expiration dates of their terms are:

R. L. Bernard, USDA (1982)  
Turner Hall  
Dept. of Agronomy  
University of Illinois  
Urbana, IL 61801

H. R. Boerma (1980)  
Dept. of Agronomy  
University of Georgia  
Athens, GA 30602

T. E. Devine, USDA (1982)  
CCNFL, Bldg. 001  
BARC-West  
Beltsville, MD 20705

T. Hymowitz (1981)  
Dept. of Agronomy  
University of Illinois  
Urbana, IL 61801

T. C. Kilen, USDA (1980)  
Delta Branch Exp. Station  
Soybean Prod. Res.  
Stoneville, MS 38776

R. G. Palmer, USDA  
(Editor of Soybean Genetics  
Newsletter)  
Dept. of Genetics  
Iowa State University  
Ames, IA 50011

J. R. Wilcox, Chm., USDA (1981)  
Dept. of Agronomy  
Purdue University  
West Lafayette, IN 47907

B) Organization of the Committee:

- 1) The Committee will be composed of six elected members and the editor of the Soybean Genetics Newsletter.
- 2) The term of the elected members will be three years. After a member has been off for one year, he (she) can be reelected. The Committee will elect two new members each year; a simple majority is needed for election. The members will be elected prior to February 1 of each year, by a mail ballot conducted by the chairman.
- 3) At the annual meeting of the Committee (usually in February in conjunction with the Soybean Breeding and Genetics Workshop), the two new members and the two retiring members of the Committee are eligible to attend and vote.
- 4) The Chairman will be elected at the annual Committee meeting and serve through the next annual meeting, and may be reelected.

C) The duties of this Committee were reviewed at Ames, IA, March 13 and 15, 1979, and the following procedures were approved:

1) Maintain Genetic Collection.

The Genetic Collection is divided into four categories:

- a) Type Collection includes all published genes of soybeans, preferably in the original strains (excluding U.S. and Canadian name varieties, which are maintained in a separate collection) plus certain mutants or strains that appear to the Committee to have potential genetic interest.
- b) Isoline Collection includes adapted varieties Clark, Harosoy and Lee, into which have been backcrossed single genes or combinations of genes. Also included are certain genes or combinations with Chippewa, Wayne and Williams.
- c) Linkage Collection includes linkage combinations and the various genetic recombinations.
- d) Cytological Collection includes translocations, inversions, deficiencies, trisomics, tetraploids, etc.

Collections a, b and c are maintained at Urbana, Illinois, with R. L. Bernard as curator. Collection d is maintained at Ames, Iowa, with R. G. Palmer as curator.

D) Manuscript review and genetic symbol approval.

The Soybean Genetics Committee requests that researchers submit all manuscripts concerning qualitative genetic interpretation and symbols to the Committee Chairman. This review by the Genetics Committee will serve to insure orderly identification and use of genetic nomenclature and to avoid conflict of symbols. This will also allow assignment of type collection designations (T-numbers) prior to publication, so that these T-numbers may be used in the journal article to identify parental lines.

E) Soybean Genetics Newsletter notes.

All notes for the Newsletter should be sent to the SGN editor, R. G. Palmer, who will ask the Soybean Genetics Committee to review those articles concerning qualitative genetic interpretation and symbols. Genetic symbols reported in the Newsletter will have the same status as those published in scientific journals.

## Rules for Genetic Symbols

## I) Gene Symbols

- a) A gene symbol shall consist of a base of one to three letters, to which may be appended subscripts and/or superscripts as described below.
- b) Genes that are allelic shall be symbolized with the same base letter(s) so that each gene locus will be designated by a characteristic symbol base.
- c) The first pair of genes reported for a gene locus shall be differentiated by capitalizing the first letter of the symbol for the dominant or partially dominant allele. (Example: Ab, ab. Ab is allelic and dominant to ab.) If genes are equivalent, codominant, or if dominance is not consistent, the capitalized symbol may be assigned at the author's discretion.
- d) When more than two alleles exist for a locus, the additional alleles or those symbolized subsequently to the pair first published shall be differentiated by adding one or two uncapitalized letters as a superscript to the base. (Example: R, r<sup>m</sup>, r.) This shall be the only use of superscripts. The base for the additional alleles is capitalized only when the gene is dominant or equivalent to the allele originally designated with a capitalized symbol. The superscript may be an abbreviation of a descriptive term. When allelism is discovered for a gene previously assigned a symbol, the previous symbol may be used as the superscript.
- e) Gene pairs with the same or similar effects (including duplicate, complementary, or polymeric genes) should be designated with the same letter base differentiated by numerical subscripts, assigning 1, 2, 3, 4, etc., consecutively in the order of publication. (Example: The y series for chlorophyll deficiency.) This shall be the only use of subscripts. Letter subscripts should not be used. The subscript 1 is automatically a part of the first reported gene symbol for each base but may be omitted until the second symbol is assigned.
- f) Base letters may be chosen so as to indicate apparent relationships among traits by using common initial letters for all loci in a related

group of traits. Examples are P for pubescence type, R for disease reaction (plus two initials of the pathogen to complete the base), and L for leaf shape.

- g) The distinction between traits that are to be symbolized with identical, similar, or with unrelated base letters is necessarily not clear cut. The decision for intermediate cases is at the discretion of the author but should be in accordance with previous practices for the particular type of trait. The following sections concern supplementary symbols that may be used whenever desired as aids to presentation of genetic formulas.
- h) A dash may be used in place of a gene symbol to represent any allele at the indicated locus. The locus represented should be apparent from its position in the formula. (Example: A represents both AA and Aa.)
- i) A question mark may be used in place of a symbol when the gene is unknown or doubtful, or it may be used as a superscript to the base symbol for the same purpose. (Example: a<sup>?</sup> indicates that the latter is an unknown allele at the A locus.)
- j) Plus symbols may be used in place of the assigned gene symbols of a designated standard homozygous strain when this will facilitate presenting genetic formulas. The standard strain may be any strain selected by the worker, as long as the strain being used and its genetic formula are made explicit.

## II) Linkage and Chromosome Symbols

- a) Linkage groups and the corresponding chromosomes shall be designated with Arabic numerals. Linkage shall be indicated in a genetic formula by preceding the linked genes with the linkage group number and listing the gene symbols in the order that they occur on the chromosome.
- b) Permanent symbols for chromosomal aberrations shall include a symbol denoting the type of aberration plus the chromosome number(s) involved. Specific aberrations involving the same chromosome(s) shall be differentiated by a letter as follows: The symbol Tran shall denote translocations. Tran 1-2a would represent the first case of reciprocal translocations between chromosomes 1 and 2, Tran 1-2b the second, etc.



The symbol Def shall denote deficiencies, Inv inversions, and Tri primary trisomics. The first published deficiency in chromosome 1 shall be symbolized as Def 1a, the second as Def 1b, etc. The first published inversion in chromosome 1 shall be denoted as Inv 1a, etc. The first published primary trisomic shall be designated with the Arabic numeral that corresponds to its respective linkage group number.

- c) Temporary symbols for chromosomal aberrations are necessary, as it may be many years before they are located on their respective chromosomes. Tran 1 would represent the first case of a published reciprocal translocation; Tran 2, the second case, etc. The first published deficiency shall be symbolized as Def A, the second as Def B, etc. The first published inversion shall be symbolized as Inv A, the second as Inv B, etc. The first published primary trisomic shall be designated as Tri A, the second as Tri B, etc. When appropriate genetic and/or cytological evidence is available, the temporary symbols should be replaced with permanent symbols, with the approval of the Soybean Genetics Committee.

### III) Cytoplasmic Factor Symbols

- a) Cytoplasmic factors shall be designated with one or more letters prefixed by cyt-. (Example: cyt-G indicates the cytoplasmic factor for maternal green cotyledons, cyt-Y indicates that for maternal yellow cotyledons.)

### IV) Priority and Validity of Symbols

- a) A symbol shall be considered valid only when published in a recognized scientific journal, or when reported in the Soybean Genetics Newsletter, with conclusions adequately supported by data which establish the existence of the entity being symbolized. Publication should include an adequate description of the phenotype in biological terminology, including quantitative measurements wherever pertinent.
- b) In cases where different symbols have been assigned to the same factor, the symbol first published should be the accepted symbol, unless the original interpretation is shown to be incorrect, the symbol is not in accordance with these rules, or additional evidence shows that a change is necessary.

### V) Rule Changes

- a) These rules may be revised or amended by a majority vote of the Soybean Genetics Committee.

IV. SYMBOLS AT THE Rps<sub>1</sub> LOCUS

Some confusion may have resulted from the publication and use of various symbols for the several alleles at the Rps<sub>1</sub> locus. The Soybean Genetics Committee recognizes the following gene symbols with respect to the Rps<sub>1</sub> locus:

Accepted gene symbol	Source	Reference
<u>rps<sub>1</sub></u>	Harosoy	Bernard (as <u>ps</u> ), Hartwig (as <u>rps<sub>1</sub></u> )
<u>Rps<sub>1</sub></u>	Mukden	Bernard (as <u>Ps</u> ), Hartwig (as <u>Rps<sub>1</sub></u> ), Mueller (as <u>Rps<sup>a</sup></u> )
<u>Rps<sub>1</sub><sup>b</sup></u>	FC 31745; PI 84,637	Hartwig (as <u>rps<sub>1</sub><sup>2</sup></u> ), Mueller (as <u>Rps<sup>b</sup></u> )
<u>Rps<sub>1</sub><sup>c</sup></u>	Arksoy; PI 54,615-1	Lam-Sanchez (as <u>Rps<sub>1</sub></u> ), Mueller (as <u>Rps<sup>c</sup></u> )

References

- Bernard, R. L., P. E. Smith, M. J. Kaufmann and A. F. Schmitthenner. 1957. Inheritance of resistance to Phytophthora root and stem rot in the soybean. *Agron. J.* 49: 391.
- Hartwig, E. E., B. L. Keeling and C. J. Edwards, Jr. 1968. Inheritance of reaction to Phytophthora rot in soybean. *Crop Sci.* 8: 634-635.
- Lam-Sanchez, A., A. H. Probst, F. A. Laviolette, J. F. Schafer and K. L. Athow. 1968. Sources and inheritance of resistance to Phytophthora megasperma var. sojae in soybeans. *Crop Sci.* 8: 329-330.
- Mueller, E. H., K. L. Athow and F. A. Laviolette. 1978. Inheritance of resistance to four physiologic races of Phytophthora megasperma var. sojae. *Phytopathology* 68: 1318-1322.

## V. GENETIC STOCKS AVAILABLE

Table 1

Recent additions to the Soybean Genetic Type Collection List<sup>†</sup>

Strain	Genes or description	Source	Maturity	Code	Reference Soybean Genet. News1.
T263	<u>df<sub>5</sub></u>	Found in Harosoy 63 x PI 257,435 in the Iowa State University nursery in Hawaii. A74-2	II	PGNBr DYY	1977 4: 40-42
(T264 to T268H, see <sup>†</sup> )					
T269H	<u>Fs<sub>1</sub>fs<sub>1</sub>fs<sub>2</sub>fs<sub>2</sub></u>	Flower structure mutant found segregating in a plant progeny row from the original PI 339,868	III	WGATn DYBf	1979 6: 57-59
T270H		Chlorophyll deficient found segregating in an F <sub>2</sub> plant progeny row from an out-cross in A76-518-3 ( <u>msp</u> <u>msp</u> )	IV	PTNBr DYB1	1979 6: 52-53
T271H	<u>msp</u>	Partial male sterile found in germplasm population AP6(S1)C1 at Iowa State University in 1975	II	PTNBr DYB1	1979 6: 47-49
T272H	<u>st<sub>5</sub></u>	Found in Uniform Test entry W6-4108 in 1970 at Ames, IA. A71-44-13	I	WTNBr DYB1	1979 6: 59-62
T273H	<u>ms<sub>3</sub></u>	Semi-sterile plant found in F <sub>3</sub> -derived line from Calland x Cutler in 1971 at Washington, IA. A72-1711	IV	PTNBr DYB1	1979 6: 63-64
T274H	<u>ms<sub>4</sub></u>	Semi-sterile plant found in cultivar 'Rampage' in 1973 at Ames, IA. A74-4646	I	PTNBr SYB1	1979 6: 64-66
	<u>rpv<sub>2</sub></u>	Resistance to peanut mottle virus. Arksoy, Peking, PI 89,784, PI 219,789			1978 5: 97-100

<sup>†</sup>For additional information see Soybean Genetics Newsletters 1976 3: 62-67 and 1977 4: 82.

## VI. USDA SOYBEAN GERmplasm REPORT

We would like to use the Soybean Genetics Newsletter as a means of making an annual report on the USDA soybean germplasm collection at Urbana, Illinois, U.S.A.

In September 1978, Randall L. Nelson accepted a position of research geneticist with the USDA and has been assigned to work with the soybean germplasm collection at Urbana. His major duties will be evaluation and utilization of the collection and, with your cooperation, compilation of data already collected on the soybean germplasm.

We will be contacting many of you by letter requesting information which your research has provided concerning the germplasm collection. If we are unable to contact you by letter and you have information you would like to share, it would be most welcome. Please include the character(s) studied, the lines which were screened, the years during which the research was conducted, the method(s) used in screening and the system of scoring, if applicable, and the results of the work. References to publications involving the germplasm collection are also being solicited.

The germplasm collection at Urbana has almost doubled in size in the last five years. The collection now includes 6015 lines. A breakdown of this material is given in Table 1.

The PI collection includes 427 lines (PI 423,973 - PI 424,617) which were added after the harvest of 1978. These were obtained from China (3), Hungary (4), Japan (15), South Korea (390), Poland (1), and Yugoslavia (14). Approximately 90% of the lines came from South Korea where primitive land races are still widely grown in gardens and small plots. The large number of recent additions from Korea are due to the work and cooperation of Dr. Shin Han Kwon of the Korean Atomic Energy Institute in Seoul and Dr. Keun Yong Park of the Office of Rural Development, Crop Experiment Station, in Suweon, who have thoroughly collected in South Korea and have generously made this material available to us.

Table 1  
Material in the USDA soybean germplasm collection  
at Urbana (January 1, 1979)

Designation	Number of lines
PI collection	4582
FC collection	51
Isolines	300
Type collection	101
Domestic varieties	252
<u>Glycine soja</u>	558
<u>Perennial species</u>	<u>171</u>
Total	6015

Also listed in Table 2 are 69 lines which are later in maturity than Group IV. These lines have been sent to the southern germplasm collection at Stoneville, Mississippi.

The collection of Glycine soja has been increased by 180 lines this year (PI 423,988 - PI 424,130). These accessions were obtained from Siberia (19) and Korea (161).

As the germplasm collection has grown, so has the distribution of the seed from the collection. Table 3 summarizes the distribution from Urbana for the last four years and for 1970. In addition, approximately 75 requests are filled each year for researchers at the University of Illinois.

More information and/or seeds from the collection may be obtained by writing to Dr. R. L. Bernard, Department of Agronomy, Turner Hall, University of Illinois, Urbana, Illinois 61801, U.S.A.

Table 2  
Maturity group listing of the latest additions  
to the soybean collection

Maturity group	Number of lines
0	12
I	9
II	3
III	30
IV	373
V and VI	69
Total	496

Table 3  
Distribution of material from the soybean  
germplasm collection at Urbana

Year	Number of requests	Number of lots distributed	States within the USA requesting seeds	Other countries requesting seeds
1970	155	9,000	31	7
1975	200	8,000	30	23
1976	250	18,000	36	25
1977	250	11,000	37	23
1978	250	10,000	32	22

Randall L. Nelson—USDA  
Richard L. Bernard—USDA



## VII. RESEARCH NOTES

NEW SOUTH WALES DEPARTMENT OF AGRICULTURE  
Agricultural Research Station, P.M.B.  
Myall Vale, Narrabri, NSW, Australia 2390

1) Resistance to Heliothis armigera and Heliothis punctigera in three soybean lines.

Multiple insect resistance has been detected in the soybean lines PI 171,451, PI 227,687 and PI 229,358 (Van Duyn et al., 1971; Clark et al., 1972; Hatchett et al., 1976). In particular Hatchett et al. (1976) demonstrated resistance to Heliothis zea and Heliothis virescens in all three lines. On the basis of percentage larval mortality, PI 171,451 and PI 229,358 were more resistant to H. zea than H. virescens, while PI 227,687 was equally resistant to both species and also showed superior levels of resistance.

A laboratory feeding trial was conducted to determine if these three soybean genotypes were also resistant to the Australian Heliothis species, H. armigera and H. punctigera.

Materials and Methods: Seedlings of PI 171,451, PI 227,687, PI 229,358 and 'Bragg' were grown in the glasshouse. When they reached the second trifoliate stage individual leaves and newly hatched larvae were placed in petri dishes. The cultures were maintained in a controlled environment room at 28°C. Leaves were replenished as necessary from the same plants for the duration of the test.

Thirty six larvae of H. punctigera and 18 larvae of H. armigera were tested on each plant genotype. Larval weight, mortality, pupal weight and time to pupation were recorded.

Results and Discussions: All three resistant genotypes have greater resistance to both Heliothis species than does Bragg. All three were also more effective against H. punctigera than H. armigera in terms of total mortality but this was not evident in the larval weight data.

Larval weights at day 11 are listed in Table 1. Larvae of H. armigera were 3-4 times larger than those of H. punctigera. This indicates that "non-resistant" soybean genotypes have an inhibiting effect on growth of H. punctigera, since larvae of both species fed on artificial diet are of approximately equal weight. Larvae fed on PI 227,687 were smaller than for the other resistant types suggesting a greater level of resistance for this genotype especially to H. punctigera.

Larval mortality occurred at two distinct times. There was considerable mortality in the first four to six days of feeding particularly for H. punctigera. A second incidence of mortality occurred just prior to pupation in both species. The mortality figures listed in Table 2 show that all three resistant genotypes were effective against H. punctigera. PI 227,687 was superior to the other genotypes in its resistance to H. armigera.

Time of pupation was lengthened for the surviving larvae on the resistant genotypes compared with Bragg. For H. armigera pupation on the resistant genotypes was delayed by four days. The H. punctigera larvae which survived on PI 171,451 were delayed by four days compared with Bragg.

Table 1

Mean weights (mg) of surviving larvae of H. armigera and H. punctigera after 11 days growth on four soybean genotypes

Genotype	Insect species	
	<u>H. armigera</u>	<u>H. punctigera</u>
PI 171,451	587	249
PI 227,687	489	88
PI 229,358	876	211
Bragg	1277	387

Table 2

Percent mortality of H. armigera and H. punctigera at day 11 and pupation for four soybean genotypes

Genotype	Insect species	
	<u>H. armigera</u>	<u>H. punctigera</u>
----- Day 11 -----		
PI 171,451	6	36
PI 227,687	6	65
PI 229,358	12	62
Bragg	0	12
----- Pupation -----		
PI 171,451	28	89
PI 227,687	56	100
PI 229,358	39	100
Bragg	6	56

Hatchett et al. (1976) found no larvae of H. zea or H. virescens survived on PI 227,687 and that this line may have a different genetic basis for resistance than the other two lines. In this trial, the resistance shown by PI 227,687 was the most effective although some larvae of H. armigera did reach pupation. The use of PI 227,687 in breeding for resistance to H. armigera and H. punctigera would be expected to be effective.

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## 2) Response of four soybean varieties to foliar zinc fertilizer.

Zinc deficiency symptoms are commonly encountered in irrigated soybean crops grown on grey self-mulching clay soils in Northern N.S.W. These experiments aimed to (1) quantify the yield loss due to zinc deficiency at different sites and (2) examine the differences in genotypic sensitivity to zinc deficiency among commercially grown soybean cultivars.

Materials and Methods: Experiments were conducted at (1) Narrabri Agricultural Research Station, (2) Breeza Substation and (3) Trangie Agricultural Research Station. Zinc fertilizer was applied as a foliar spray of  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  at each site prior to flowering. Rate of zinc application was 4Kg/ha of Zn at Narrabri and Trangie and 8Kg/ha in two sprays of 4Kg/ha each at Breeza.

At each site the experimental design was a split plot with zinc treatments as main plots. The four commercial soybean cultivars 'Bragg', 'Lee', 'Forrest' and 'Dodds' were sown as subplots. All sites were irrigated as required, and weeds and insects controlled.

Results and Discussion: Yields for +Zn and nil Zn treatments are listed in Table 1. Response to zinc differed across sites and among varieties within each site.

Lee was the variety that showed least response to applied zinc at all three sites. However, the most responsive variety differed among sites with Dodds, Bragg and Forrest giving the greatest yield increase at Narrabri, Trangie and Breeza respectively.

The Narrabri site gave the lowest responses but these increases in yield were economically and statistically significant. The responses at this site were obtained in the absence of visible foliar symptoms of Zn deficiency.

A variety trial in an adjacent area within the same field at Breeza received an additional application of  $\text{ZnSO}_4$  during ground preparation. In that trial Bragg, Forrest, Dodds and Lee yielded 3685, 3542, 3364 and 3172 Kg/ha respectively.

Table 1  
Yield response (kg/ha) of four varieties at three  
sites with applied zinc

Variety	Zinc	Site		
		Narrabri	Trangie	Breeza
Bragg	+	3347	2106	2494
	Nil	3013	1161	549
Lee	+	2798	1771	2640
	Nil	2768	1714	1707
Forrest	+	3610	1002	2471
	Nil	3139	677	322
Dodds	+	3329	1192	2161
	Nil	2678	920	583
l.s.d. (0.05)				
Variety x Zinc means		411	309	564

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### 1) Soybean linkage tests.

F<sub>2</sub> linkage results are presented in Table 1 with a = XY, b = Xy, c = xY and d = xy for the eight gene pairs listed in the form of Xx and Yy. Percentage recombination was obtained from the ratio products following Immer and Henderson (1943). The data for each of the gene pairs gave a good fit to a 3:1 ratio.

Rps<sub>1</sub>/rps<sub>1</sub> was evaluated using race 1 of Phytophthora megasperma var. sojae in hypocotyl tests of F<sub>3</sub> seedlings. Rmd/rmd was evaluated for adult plant resistance and susceptibility to powdery mildew using greenhouse inoculation of F<sub>3</sub> progenies with Microsphaera diffusa.

The previously reported possibility that Rmd/rmd is in Linkage Group 1 (Buzzell, 1978) was supported by the linkage of Fg<sub>3</sub>/fg<sub>3</sub> with Rmd/rmd. Fg<sub>3</sub>/fg<sub>3</sub> is between T/t and Rmd/rmd but is closer to T/t. A combined estimate using data from 'Blackhawk' x 'Kingwa' (Buzzell, 1977) and Table 1 indicates 13.6 ± 4.4% recombination between Fg<sub>3</sub>/fg<sub>3</sub> and T/t.

Table 1  
Soybean F<sub>2</sub> linkage tests  
Blackhawk (Rps t w<sub>1</sub> l<sub>1</sub> fg<sub>1</sub> fg<sub>2</sub> Fg<sub>3</sub> Rmd) x PI 65,388 (rps<sub>1</sub> T W<sub>1</sub> L<sub>1</sub> Fg<sub>1</sub> Fg<sub>2</sub> fg<sub>3</sub> rmd)

Genes		a	b	c	d	%R	SE	Phase
Rps <sub>1</sub> rps <sub>1</sub>	T t	168	44	48	16	53.2	4.4	R
	W <sub>1</sub> w <sub>1</sub>	169	48	40	17	> 55	-	R
	L <sub>1</sub> l <sub>1</sub>	153	64	42	17	44.5	4.5	R
	Fg <sub>1</sub> fg <sub>1</sub>	160	56	44	14	48.4	4.6	R
	Fg <sub>2</sub> fg <sub>2</sub>	167	49	41	17	54.8	4.3	R
	Fg <sub>3</sub> fg <sub>3</sub>	161	56	44	14	51.2	4.6	C
	Rmd rmd	164	51	50	7	> 55	-	C
Rmd rmd	T t	171	45	48	10	46.7	4.7	R
	W <sub>1</sub> w <sub>1</sub>	163	53	46	12	47.0	4.7	R
	L <sub>1</sub> l <sub>1</sub>	156	60	39	19	53.2	4.4	R
	Fg <sub>1</sub> fg <sub>1</sub>	159	55	46	13	47.2	4.7	R
	Fg <sub>2</sub> fg <sub>2</sub>	163	50	46	14	49.9	4.5	R
	Fg <sub>3</sub> fg <sub>3</sub>	167	51	34	23	39.1	3.9	C
T t	W <sub>1</sub> w <sub>1</sub>	171	49	36	16	45.0	4.3	C
	Fg <sub>1</sub> fg <sub>1</sub>	157	64	50	7	> 55	-	C
	Fg <sub>2</sub> fg <sub>2</sub>	164	57	48	10	> 55	-	C
	Fg <sub>3</sub> fg <sub>3</sub>	150	70	56	1	13.5	5.9	R
L <sub>1</sub> l <sub>1</sub>	T t	161	36	61	21	44.0	4.2	C
	W <sub>1</sub> w <sub>1</sub>	148	47	62	18	50.5	4.5	C
	Fg <sub>1</sub> fg <sub>1</sub>	146	48	61	22	48.7	4.4	C
	Fg <sub>2</sub> fg <sub>2</sub>	151	44	59	23	45.9	4.3	C
	Fg <sub>3</sub> fg <sub>3</sub>	146	50	61	22	50.6	4.5	R
Fg <sub>1</sub> fg <sub>1</sub>	W <sub>1</sub> w <sub>1</sub>	155	48	53	17	49.5	4.5	C
	Fg <sub>2</sub> fg <sub>2</sub>	163	50	53	17	49.3	4.4	C
	Fg <sub>3</sub> fg <sub>3</sub>	157	56	53	17	48.5	4.5	R
Fg <sub>2</sub> fg <sub>2</sub>	W <sub>1</sub> w <sub>1</sub>	161	46	47	18	45.8	4.3	C
	Fg <sub>3</sub> fg <sub>3</sub>	165	51	44	23	> 55	-	R
Fg <sub>3</sub> fg <sub>3</sub>	W <sub>1</sub> w <sub>1</sub>	154	49	53	17	50.1	4.5	R

N = 272-283.

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2) Soybean parental lines.

Seed of five improved breeding lines (Table 1) is available upon request for use in crosses or experimental work. Disease reactions are given in Table 2 and physiological attributes in Table 3.

The lines are somewhat improved over the unadapted parents; however, only OX610I has been yield tested. It yielded 16% less than 'Harcor' over 3 locations in 1978, but its yield and photosynthetic rate could have been reduced by leafhopper damage. Apparently the pubescence on OX610I is less dense than normal.

Table 1  
Origin and description of parental lines

Line	Origin	Color		
		Flower	Hilum	Pubescence
OX298	Harwood x Toyosuzu	Purple	Yellow	Gray
OX610I	Harwood x Tokachishiro	Purple	Yellow	Gray
OX615	Harcor <sup>2</sup> x Raiden	Purple	Yellow	Gray
OX693	Harosoy 63 x Altona	Purple	Black/Brown	Brown
OX696	Harosoy x Kingwa	Purple	Yellow*	Gray

\*Seed are yellow/green.

Table 2  
Disease reactions of parental lines

Phytophthora megasperma var. sojae										
Line	% plant loss*	Hypocotyl reaction** to races							PM	SMV
		1	2	3	4	5	6-7	8-9		
OX298	-	R	R	R	R	R	R	R	S	S
OX610I	-	R	R	S	S	S	R	R/S	RJ	-
OX615	-	R	R	R	S	S	R	R	S	R
OX693	-	R	R	R	R	S	S	S	RJ	S
OX696	-	R	R	R	R	R	R	R	RA	-
Harcor	17	R	R	S	S	S	S	S	S	S
Harosoy 63	49	R	R	S	S	S	S	S	S	S

\*Average of 1977-78 in an infested field (races 3, 7 and 9).

\*\*R= resistant; S= susceptible.

PM= powdery mildew caused by Microsphaera diffusa. RJ= juvenile and adult resistance; RA= adult resistance.

SMV= soybean mosaic virus (race or races unknown). R= resistance to leaf symptoms and seedcoat mottling; appears to be controlled by a single dominant gene.

Table 3  
Physiological characteristics of parental lines

Line	Days to mature	Plant ht, cm	Leaflet* area, cm <sup>2</sup>	SLW** mg/cm <sup>2</sup>	Chlorophyll** mg/dm <sup>2</sup>	P <sub>A</sub> ** mgCO <sub>2</sub> /dm <sup>2</sup> /h
OX298	121	66	87	5.6	3.8	28
OX610I	124	75	86	5.2	3.7	24
OX615	114	80	72	5.4	4.1	31
OX693	104	57	72	6.8	4.3	31
OX696	128	72	78	4.6	3.6	25
Harcor	125	78	82	5.4	3.9	28
Harosoy 63	122	81	94	5.2	3.7	25
L.S.D. 0.05	-	-	ns	1.19	0.68	5.5
C.V. %	-	-	12.4	9.6	8.1	16.5

\*Most recently fully-expanded leaves sampled July 26; 9 per plot in 4 replicates.

\*\*Average of 6-replicate determinations July 28 and August 16, 1978.

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1) Genetic analysis of factors controlling nodulation response in soybeans.

Two of the genes controlling nodulation response in soybeans were tested for linkage associations with genes controlling pubescence color (T) and flower color (W<sub>1</sub>), chlorophyll deficiency (y<sub>9</sub>) and absence of pubescence (P). The rj<sub>1</sub> gene (Williams and Lynch, 1954) in homozygous recessive condition results in a non-nodulating phenotype with a broad spectrum of Rhizobium japonicum strains. The dominant gene, Rj<sub>4</sub> (Vest and Caldwell, 1972) conditions an ineffective nodulation response when inoculated specifically with R. japonicum strain 61 of the Beltsville Culture Collection.

Genetic stocks (T lines) and Clark rj<sub>1</sub> rj<sub>1</sub> were obtained from the Soybean Genetic Type Collection (Bernard and Weiss, 1973). Crosses were made in the field and F<sub>1</sub> seed were advanced to the F<sub>2</sub> generation in the greenhouse. F<sub>3</sub> seed was produced in the field at Beltsville. F<sub>3</sub> progeny rows derived from individual F<sub>2</sub> plants of rj<sub>1</sub> crosses were evaluated for phenotype in the field at Beltsville. Crosses with Rj<sub>4</sub> were evaluated in plastic growth tray assemblies (Devine and Reisinger, 1978) and inoculated with 7-day-old broth cultures of R. japonicum strain 61. F<sub>2</sub> genotypes were rationalized from F<sub>3</sub> phenotypes. Results of these linkage tests (Table 1) indicate independent assortment of rj<sub>1</sub> and T, rj<sub>1</sub> and W<sub>1</sub>, rj<sub>1</sub> and P, and Rj<sub>4</sub> and y<sub>9</sub>. A linkage association is apparent between Rj<sub>4</sub> and P in linkage group 2. Reasoning from this

Table 1  
Soybean Linkage Test

Genes	a	b	c	d	Sum	%R*	SE	Phase
Clark <u>rj<sub>1</sub></u> ( <u>rj<sub>1</sub></u> <u>rj<sub>1</sub></u> <u>T</u> <u>T</u> <u>W<sub>1</sub></u> <u>W<sub>1</sub></u> ) x Hardee ( <u>Rj<sub>1</sub></u> <u>Rj<sub>1</sub></u> <u>t</u> <u>t</u> <u>w<sub>1</sub></u> <u>w<sub>1</sub></u> )								
<u>Rj<sub>1</sub></u> <u>rj<sub>1</sub></u> <u>T</u> <u>t</u>	110	41	37	10	198	46	5	R
<u>Rj<sub>1</sub></u> <u>rj<sub>1</sub></u> <u>W<sub>1</sub></u> <u>w<sub>1</sub></u>	107	40	36	9	192	44	8	R
Clark <u>rj<sub>1</sub></u> ( <u>rj<sub>1</sub></u> <u>rj<sub>1</sub></u> <u>W<sub>1</sub></u> <u>W<sub>1</sub></u> ) x Hill ( <u>Rj<sub>1</sub></u> <u>Rj<sub>1</sub></u> <u>w<sub>1</sub></u> <u>w<sub>1</sub></u> )								
<u>Rj<sub>1</sub></u> <u>rj<sub>1</sub></u> <u>W<sub>1</sub></u> <u>w<sub>1</sub></u>	115	40	36	13	204	50	-	R
T135 ( <u>rj<sub>4</sub></u> <u>rj<sub>4</sub></u> <u>y<sub>9</sub></u> <u>y<sub>9</sub></u> ) x Hill ( <u>Rj<sub>4</sub></u> <u>Rj<sub>4</sub></u> <u>Y<sub>9</sub></u> <u>Y<sub>9</sub></u> )								
<u>Rj<sub>4</sub></u> <u>rj<sub>4</sub></u> <u>Y<sub>9</sub></u> <u>y<sub>9</sub></u>	119	37	27	13	196	44	6	C
Hill ( <u>Rj<sub>4</sub></u> <u>Rj<sub>4</sub></u> <u>p</u> <u>p</u> ) x T145 ( <u>rj<sub>4</sub></u> <u>rj<sub>4</sub></u> <u>P</u> <u>P</u> )								
<u>Rj<sub>4</sub></u> <u>rj<sub>4</sub></u> <u>P</u> <u>p</u>	125	66	47	9	244	36	5	R
T145 ( <u>Rj<sub>1</sub></u> <u>Rj<sub>1</sub></u> <u>P</u> <u>P</u> ) x Clark ( <u>rj<sub>1</sub></u> <u>rj<sub>1</sub></u> <u>p</u> <u>p</u> )								
<u>Rj<sub>1</sub></u> <u>rj<sub>1</sub></u> <u>P</u> <u>p</u>	115	33	34	9	191	51	5	C

\*Recombination percentages calculated by the product method (Immer and Henderson, 1943).

positive linkage association and the lack of linkage of P with rj<sub>1</sub>, we conclude that Rj<sub>4</sub> and rj<sub>1</sub> are not allelic.

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## 2) Significance of incompatibility reactions of *Rhizobium japonicum* strains with soybean host genotypes.

Soybeans normally nodulate with *Rhizobium japonicum* and fix nitrogen in symbiotic association. However, several interactions, under genetic control, have been reported which result in ineffective nodulation or failure of the fixation process despite substantial nodule development. The recessive gene, *rj*<sub>1</sub>, (Williams and Lynch, 1954) in homozygous mode, results in the exclusion from nodulation of a broad spectrum of *Rhizobium japonicum* strains in soil culture. No evidence of nodule development is visible to the unaided eye. The *Rj*<sub>2</sub> gene, a dominant factor, reported in the cultivars 'Hardee' and 'CNS' (Caldwell, 1966), results in the formation of cortical proliferations or rudimentary nodules when plants are inoculated with *R. japonicum* strains of the c1 or 122 serogroup.

The gene *Rj*<sub>3</sub>, also reported in the cultivar Hardee (Vest, 1970), produces an ineffective nodulation reaction specifically with *Rhizobium* strain 33 of the Beltsville Culture Collection. The *Rj*<sub>4</sub> gene, reported in the cultivar 'Hill' (Vest and Caldwell, 1972), conditions ineffective nodule development, specifically with *R. japonicum* strain 61. Another type of incompatible reaction occurs when the cultivar 'Peking' is inoculated with *R. japonicum* strain 123 (Vest et al., 1972). Nodules are formed in normal frequency and size. However, virtually no nitrogen is fixed. Several other *Rhizobium* strains exhibit varying degrees of inefficiency in fixation with Peking.

These aberrant reactions have been regarded as interesting but troublesome biological oddities. The literature provides no explanation for their occurrence. Two hypotheses are proposed here.

First, the *Rj* genes may be "inborn metabolic errors" (analogous to phenylketonuria in man), which arose by mutation in plant breeders' stocks and have not (Devine, 1976) been eliminated from breeders' lines. Second, the incompatible reactions may result from coupling genotypes of the host and microsymbiont which have not coevolved in the same locality. Natural selection would have occurred for mutual compatibility during coevolution in Asia. The reassortment of germplasm of host and microsymbiont occurring with introduction to the New World may have resulted in association of ecotypes alien to each other, resulting in incompatible reactions.

To test these hypotheses, Plant Introductions (PI's) representing several countries and maturity groups are being tested with the *Rhizobium* strains defining for the *Rj* reactions. A portion of the results of this survey is presented here.

The Plant Introductions were planted in hills of five seed each in plant growth trays (Devine and Reisinger, 1978), 24 hills per tray. Seed were surface sterilized with 50% ETOH before planting and inoculated with the strain appropriate for definition of the pertinent *Rj* factor. Plants were evaluated two or three weeks after planting. Approximately 30 PI's were sampled in each of the maturity groups I through VIII. Seven countries are represented in the sample of PI's.

The *Rj*<sub>2</sub> gene does occur in Asiatic populations, however, at a low frequency in the population sampled (Table 1). The *Rj*<sub>4</sub> gene occurs with much higher frequency (Table 2). All five PI's from Thailand carry *Rj*<sub>4</sub> as do four of the five PI's from Indonesia. These results lead to the conclusion that the first hypothesis is not tenable in the case of *Rj*<sub>2</sub> and *Rj*<sub>4</sub> and that these

Table 1  
Gene frequency of  $R_{J_4}$  in soybean Plant Introductions from China, Manchuria, Korea, Japan, Taiwan, Thailand and Indonesia

Maturity Group	Origin of Plant Introduction and frequency of lines with $R_{J_4}$ phenotype													
	China and Manchuria		Korea		Japan		Taiwan		Thailand		Indonesia		Total	
	Ratio	%	Ratio	%	Ratio	%	Ratio	%	Ratio	%	Ratio	%	Ratio	%
I	1/16	6	2/4	50	0/10	0	-	-	-	-	-	-	3/30	10
II	5/10	50	0/10	0	3/10	30	-	-	-	-	-	-	8/30	27
III	5/10	50	2/10	20	1/10	10	-	-	-	-	-	-	8/30	27
IV	10/10	100	3/10	30	2/10	20	-	-	-	-	-	-	15/30	50
V	2/10	20	3/10	30	3/10	30	-	-	-	-	-	-	8/30	27
VI	3/10	30	4/9	44	5/10	50	-	-	-	-	-	-	12/29	41
VII	4/11	40	1/6	17	3/11	27	-	-	-	-	-	-	8/28	29
VIII	-	-	-	-	4/13	31	3/5	60	5/5	100	4/5	80	16/28	57

Table 2  
Gene frequency of Rj<sub>2</sub> in soybean Plant Introductions from China, Manchuria, Korea, Japan, Taiwan, Thailand and Indonesia

Maturity Group	Origin of Plant Introduction and frequency of lines with <u>Rj<sub>2</sub></u> phenotype											
	China and Manchuria		Korea		Japan		Taiwan		Thailand		Indonesia	
	Ratio	%	Ratio	%	Ratio	%	Ratio	%	Ratio	%	Ratio	%
I	0/16	0	0/4	0	0/10	0	-	-	-	-	-	0/30
II	0/10	0	0/10	0	0/10	0	-	-	-	-	-	0/30
III	0/10	0	0/10	0	0/10	0	-	-	-	-	-	0/30
IV	0/9	0	0/10	0	1/10	10	-	-	-	-	-	1/29
V	0/10*	0	0/10	0	0/10	0	-	-	-	-	-	0/30*
VI	1/10	10	0/9	0	0/10	0	-	-	-	-	-	1/29
VII	1/11	10	0/6	0	0/13	0	-	-	-	-	-	1/30
VIII	0/2	0	-	-	1/13	1	0/5	0	0/5	0	0/5	1/30
Total												

\*Heterogeneity in one line.



genes trace to Asiatic origin rather than recent mutation in U.S. breeding stocks.

Very little information is available on the precise location in Asia from which Rhizobium strains now in the production fields of the United States originated. The frequency with which these genes occur in PI's of the maturity groups and nations sampled suggest that many of the PI's evolved in areas where there was not a significant selection pressure for compatibility with Rhizobium strain 61.

I interpret these results as supporting the concept of coevolution affecting the compatibility of host strain interactions. If the interactions affecting the efficiency of fixation, as seen in the Peking x strain 123 reaction, are analogous to the Rj<sub>2</sub> and Rj<sub>4</sub> phenomenon, it may be postulated that the efficiency of nitrogen fixation in U.S. soybean production may be improved by reassembling the ecotypic associations of soybean germplasm and Rhizobium strains as they evolved in Asia.

The high frequency with which the Rj<sub>4</sub> gene occurs in the PI's and the severity of its effect in restricting nitrogen fixation in association with Rhizobium strain 61, indicates that when breeders are evaluating PI's in soils that are nitrogen deficient, the nature of the Rhizobium strains in the field may profoundly affect the performance of the PI's. In such circumstances, if the breeders' object is to determine the full biological potential of the PI's, they should apply adequate nitrogen fertilizer to their nurseries.

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### 3) Aluminum tolerance in soybean germplasm.

Aluminum in the soil solution is a severe growth limiting factor in certain acid soils (Foy, 1964; Long and Foy, 1970). This problem is particularly serious in acid subsoils (Adams and Lund, 1966; Foy, 1964) which are difficult to lime. Recent research has centered on selecting those plant cultivars which demonstrate a degree of tolerance to soil aluminum. Research with wheat has shown that differential tolerance to aluminum (Al) is related to the region in which cultivars were bred (Foy *et al.*, 1974). Devine (1976) demonstrated that Al tolerance is a heritable trait in alfalfa and that recurrent selection was an effective breeding method for modifying Al response. The objective of our present research is to identify sources of Al tolerance in soybean germplasm and to develop efficient and precise methods to assay for tolerance. The results reported here reflect a portion of the work concerning several lines of germplasm from Korea chosen for testing because of their origin from areas reputed to have low pH soils.

Plants were evaluated in a hydroponic system using four glass tanks arranged in two replications in a growth room. Forty liters of modified 1/5 Steinburg solution containing 4 ppm Ca was added to each tank (Foy *et al.*, 1967 and 1969). An Al treatment (6 ppm Al as  $\text{AlK}(\text{SO}_4)_2$ ) was added to one tank in each replication. Solutions in all tanks were aerated continuously and adjusted daily to pH 4.5 with either 1 N HCl or 1 N NaOH as required. The weight of 30 seeds used for germination was determined on a Mettler top-loading balance. The weight per 100 seeds was calculated and is presented in Table 1. Seeds were placed in germination paper and incubated at 26°C for 72 hours in the dark. For each entry, five seedlings per treatment, per replication, were then transferred to the solution culture for 72 additional hours at the same temperature. The plants were given a 16-hour daylength at 2807 lux. Each plant was measured for primary root length (PRL), length from the primary root tip to the most recently emerged secondary root (RPS), and the length of the three longest lateral roots (LRL). In addition, from a comparison of the roots in +Al treatment and -Al treatment, each entry was assigned a visual damage score ranging from 1, least damage, to 5, most damage. The cultivars 'Perry' and 'Chief', known for their respective tolerance and susceptibility to aluminum (Foy *et al.*, 1969), were included as checks.

As a measure of an entry's ability to sustain its normal growth despite Al stress, the ratio +Al/-Al was calculated for each entry in each replication and subjected to an ANOVA. Then, to permit approximate comparisons across a series of tests, the values for the two check cultivars (Perry and Chief) were averaged to obtain a standard value for the test, and the values for the other test lines were compared to this derived standard as a percent of the test standard. A partial summary of the data appears in Table 1 and correlation values are given in Table 2.

Significant differences in Al tolerance among entries were not detected with the visual score. However, with 6 ppm Al, LRL indicated that three entries (635-4, 600-7-2 and 600-4-2) were significantly more tolerant to aluminum than the resistant check cultivar Perry. The LRL ratio (+Al/-Al) also indicated differential tolerance among entries. For example, entry 600-4-2 was significantly more tolerant than the Al-sensitive cultivar Chief. However, no significant differences between Perry and Chief were detected by the parameters measured in this test. Of the four measures for detection of differential Al tolerance, i.e., visual damage score, and the +Al/-Al ratio for PRL,

Table 1  
Aluminum toxicity solution culture test no. 12-1978

Entry No.	Weight of 100 seed	Visual damage score <sup>1</sup>	PRL		PRS		LRL			
			% of standard		% of standard		0 Al (cm)	6 ppm Al (cm)	Ratio	% of standard
635-4	20.7	2.5 a*	101 a		108 a		5.5 a	5.1 abc	.99 ab	151 abc
635-6	23.2	3.0 a	105 a		105 a		5.3 ab	4.2 a-f	.85 ab	140 abc
600-7-2	20.0	2.5 a	98 a		102 a		5.0 abc	4.7 a-d	.97 ab	173 ab
635-2	17.6	3.0 a	106 a		96 a		5.9 a	4.5 a-e	.79 ab	148 abc
635-1	23.6	3.5 a	111 a		118 a		4.7 a-d	3.3 b-g	.76 ab	114 c
600-10-1	11.2	3.5 a	95 a		66 a		2.8 d-g	1.5 g	.68 b	117 c
600-8	27.9	3.0 a	107 a		114 a		4.8 a-d	3.3 c-g	.74 ab	126 bc
600-4-2	28.8	3.0 a	107 a		116 a		4.5 a-e	4.7 a-d	1.21 a	194 a
600-6-2	10.9	4.0 a	92 a		78 a		4.4 a-e	3.4 fg	.52 b	99 c
Perry	15.3	3.0 a	--		--		4.1 a-f	2.6 efg	.73 ab	--
Chief	15.3	3.5 a	--		--		4.5 a-e	2.1 g	.54 b	--

<sup>1</sup>Scored 1 to 5: 1 = least damage; 5 = most damage.

\*Any two values having a letter in common are not significantly different at the 5% level by the Duncan's multiple range test.

Table 2  
Correlation values

Observations	Seed weight	Visual score
Visual score	-.50 NS	--
PRL as % of standard	.82**	-.28 NS
PRL at 0 ppm Al	.35 NS	-.35 NS
PRL at 6 ppm Al	.64*	-.47 NS
PRS as % of standard	.93**	-.51 NS
PRS at 0 ppm Al	-.44 NS	.14 NS
PRS at 6 ppm Al	.57 NS	-.52 NS
LRL as % of standard	.54 NS	-.75*
LRL at 0 ppm Al	.44 NS	-.51 NS
LRL at 6 ppm Al	.64*	-.78*
LRL ratio +Al/-Al	.69*	-.73*

\*Significant at the 5% level.

\*\*Significant at the 1% level.

NS = not significant.

PRS and LRL, no significant differences were found for visual damage score or PRL, while PRS and LRL differences were significant. The greatest range in variation was expressed in the +Al/-Al ratio for LRL. For this reason the LRL data are presented in more detail.

To determine the influence of seed reserves on the expression of aluminum tolerance, seed weight was tested for correlation with other measurements (Table 2). Seed weight was positively correlated ( $p < .01$ ) with PRL as percent of standard and with the PRS as percent of standard, but was not significantly correlated with the LRL as percent of standard. This suggests that seed reserves strongly influence the aluminum response of primary root growth. The correlations of seed weight with the PRL, PRS, and LRL at 0 ppm Al were not significant. But, under Al stress at 6 ppm, seed weight was significantly correlated with PRL and LRL and approaches significance with PRS. This suggests that under aluminum stress the influence of seed reserves on growth is greater than in the absence of aluminum stress. Additional correlations were made to determine the influence of the factors measured on the assignment of visual damage scores. The visual damage score was not correlated with seed weight. Nor was visual score correlated with any of the measurements at 0 ppm Al. The visual score was not correlated with PRL or PRS either at 6 ppm Al or as percent of standard, suggesting that these measurements had little or no influence on the assignment of visual ratings. However, the LRL at 6 ppm Al and as percent of standard are negatively correlated with the visual score, indicating the LRL was an important factor influencing this score.

A previous study (Devine, 1976) reported that variation in seed lots of the same cultivar produced at different locations had little effect on aluminum tolerance in comparison with effect of the genotype of the zygote. In that study conducted with adapted U.S. cultivars, seed weight within a cultivar did not vary appreciably. In this study, however, seed weight varied



10.9 to 28.8 g/100 seed, a factor of 2.6. Similar variation would be expected in screening the Soybean Germplasm Collection.

These results indicate that seed weight exerts an influence on early seedling expression of Al tolerance and caution should be used in imputing long term physiological tolerance to lines expressing tolerance at this stage.

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1) Transgressive inheritance of early maturity for breeding of extremely early soybean cultivars.

The key point to extend soybean growing area to the cool, long day length, and short-growing season regions of the high latitude is breeding of day-neutral and cool-tolerable extremely early cultivars. Several countries have already got a distinct achievement in this respect. For example, extremely early soybean cultivars of Maturity Group 00 even 000 have been developed in countries of North America and North Europe. In Heilungkiang Province of the People's Republic of China, such kind of work has also been carried out for the purpose of extending soybean production to the grassland area north of Greater Sinan Mountain. In order to obtain the extremely early varieties, crosses (Table 1) were made between early varieties of different origin to accumulate the early maturity genes.

From Table 1 we can learn that, owing to the genotypic resemblance of the parents on earliness, only a few of the crosses whose parents both originated in the Northeast of China perform transgressive inheritance in  $F_2$ , and no extremely early new strains were obtained from such crosses. Because soybean germplasms of North U.S. were mostly from Northeast China, there are also only a few crosses between varieties of these two sources performing transgressive inheritance on earliness. On the other hand, when early varieties of Northeast China were crossed with early varieties of North Europe, North Japan, and Central China, a higher proportion of crosses were observed to

Table 1  
Crosses between different early varieties  
(The Northeast Agricultural College, 1970-1976)

Sources of the two parents	No. of crosses	Crosses with transgressive inheritance of earliness in $F_2$
Northeast China with Northeast China	44	4
Central China with Northeast China	3	1
North Europe with Northeast China	11	5
North Japan with Northeast China	2	1
North America with Northeast China	12	2



perform transgressive inheritance on earliness, and many promising extremely early strains were obtained. These results show that selection following crosses between early varieties with different genotypes on earliness is an effective method to develop extremely early soybean cultivars with improved agronomic characters for high latitude and short growing season regions. It is evident that discovering the source of genes governing such extreme earliness through systematic study is the foundation of such breeding work.

Table 2

The general performance of several of the newly developed  
extremely early soybean strains  
(Harbin, China, 1976-1977)

Parents and strains	Growth period (days)	Plant height (cm)	Weight of 100 seeds (g)	Yield
Heiho 3*	110	--	--	
Funsho 12*	115	--	--	
76-1959	104	89.0	20.5	25.03% (over ck)
76-1748	103	83.3	20.0	22.64% ( " )
76-287	101	84.4	19.1	20.87% ( " )
Funsho 12*	115	85.0	21.0	
Heiho 3*	110	70.0	20.0	
76-1909	103	77.6	22.5	15.19%
Kusun*	100	60.0	20.5	
Japanese Early	95	50.0	23.8	
47-1D	92	60.0	18.5	
47-1C	90	65.0	18.0	
Logbeau (Germany)	95	52.0	19.0	
47-1D	92	60.0	18.5	
76-333	83	50.0	17.5	1870 (kg/ha)
76-331	83	45.0	18.0	1900 ( " )
76-335	85	46.0	19.0	1890 ( " )
Funsho 11*	90	50.0	20.1	
Sweden Soybean	90	60.0	17.0	
77-12	87	65.0	18.6	2321 ( " )

\*Adapted cultivar of Northeast China.

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1) Screening the USDA soybean germplasm collection for  $Sp_1$  variants.<sup>1</sup>

Orf and Hymowitz (1976) using polyacrylamide gel electrophoresis revealed that the seed protein band called "A" by Larsen and Caldwell (1968) occurs at Rf 0.36 and the seed protein band called "B" occurs at Rf 0.42 (Rf = mobility relative to a bromophenol blue dye front in a 10% polyacrylamide gel anodic system using a pH 8.3 Tris-glycine buffer). The inheritance of these proteins (although the proteins were not characterized) was reported as being controlled by two codominant alleles at a single locus (Larsen and Caldwell, 1968). Orf and Hymowitz (1976) proposed the gene symbols  $Sp_1^a$  and  $Sp_1^b$  for the electrophoretic forms that occur at Rf 0.36 and Rf 0.42, respectively.

The genus Glycine Willd. is composed of two subgenera Glycine and Soja (Moench) F. J. Herm. (Hymowitz and Newell, 1979). The subgenus Glycine comprises the soybean, Glycine max (L.) Merr., and its closest wild relative G. soja Sieb. and Zucc. Glycine gracilis Skvortz. has been described as a species morphologically intermediate between G. max and G. soja (Skvortzow, 1927), but Hermann (1962) placed it under synonymy with G. max. For this report, Glycine gracilis has been separated from G. max.

The summary of the screening data is presented in Table 1; of the 2940 Glycine max accessions tested, 2617 accessions, or 89%, had the  $Sp_1^b$  allele. In the Asia collection, the remainder category is composed of soybeans introduced into the U.S. from Afghanistan, Burma, Indonesia, Malaysia, Nepal, Pakistan, Philippines, Taiwan, Thailand, the U.S.S.R. and Vietnam. Sources by Maturity Group (00 to VIII) for the  $Sp_1^b$  allele within the Named Variety Collection are 'Flambeau' (00), 'Grant' (0), 'Anoka' (I), 'Wells' (II), 'Cloud' (III), 'Clark' (IV), 'Hill' (V), 'Davis' (VI), 'Bragg' (VII) and 'Coker Hampton 266' (VIII). Sources by Maturity Group (00 to VI) for the  $Sp_1^a$  allele within the Named Variety Collection are 'Acme' (00), 'Evans' (0), 'Steele' (I), 'Amsoy' (II), 'Chestnut' (III), 'Bonus' (IV), 'Dixie' (V) and 'Rose Non-Pop' (VI).

Of the 359 Glycine soja accessions tested, 228 accessions, or 63.5%, had the  $Sp_1^b$  allele. The Glycine soja collection is made up of introductions from China, Japan, Korea, Taiwan and the U.S.S.R. All of the 39 Glycine gracilis accessions tested had the  $Sp_1^b$  allele.

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Table 1  
Distribution of  $Sp_1$  variants in the USDA  
soybean germplasm collection\*

Collection	$Sp_1^a$	$Sp_1^b$	Mixture	Total
Asia				
Japan	26	451		477
Korea	83	334		417
China	78	725		803
India	52	167		219
Remainder	8	156		164
Europe	35	399		434
Other				
Named Varieties	36	296		332
Type Collection**	10	83	1	94
<u>Glycine soja</u> **	131	223	5	359
<u>Glycine gracilis</u>	--	39	—	39
Totals	459	2873	6	3338

\*Data taken in part from Orf, 1976, 1979; Skorupska and Hymowitz, 1977.

\*\*Type Collection 230 (T230) and five accessions of Glycine soja (PI 378,693B, PI 407,075, PI 407,080, PI 407,116 and PI 407,169) were mixtures containing both  $Sp_1^a$  and  $Sp_1^b$  seed.

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## 2) Soybean linkage test between Ti and Le seed proteins.

The  $F_2$  linkage results between the Ti locus and Le locus are presented in Table 1. In the table  $a = \underline{\text{Ti}} \underline{\text{Le}}$ ,  $b = \underline{\text{Ti}} \overline{\text{Le}}$ ,  $c = \underline{\text{ti}} \underline{\text{Le}}$  and  $d = \underline{\text{ti}} \overline{\text{le}}$ . The parents used in the cross were in repulsion phase. Percentage recombination was obtained from the ratio of products following Immer and Henderson (1943).

The Ti and Le genotypes were determined using previously described procedures (Orf and Hymowitz, 1979; Orf et al., 1978). The Ti gene controls the Kunitz trypsin inhibitor and the Le gene controls a seed lectin. The results indicate these two genes are not linked.

Table 1  
Soybean  $F_2$  linkage test of Ti and Le from the cross  
PI 196,168 (ti Le) x 'Norredo' (Ti<sup>a</sup> le)

a	b	c	d	Sum	%R
59	17	15	5	96	I

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### 3) Variation in percent seed oil in related nodulating and non-nodulating $F_2$ plants and $F_3$ progenies from three soybean crosses.

The soybean (*Glycine max* [L.] Merrill) uses both combined nitrogen from the soil and symbiotically fixed nitrogen from the air if effective nodules are present on its roots. Both sources of nitrogen are required for maximum yields at reasonable costs. In the absence of nodules, yields can be brought up to the level of those where effective nodules are present, but only with a high rate of nitrogen fertilizer application. Where both systems of nitrogen utilization are operating an increase in available nitrogen in the soil usually is accompanied by a reduced activity of the nitrogen fixing bacteria (*Rhizobium japonicum*). Such behavior would seem to result in a sort of buffer action that should reduce plant-to-plant variation in nitrogen utilization as expressed by seed yield and protein percent.

Liu and Hadley (1976) reported in several crosses that phenotypic variances for seed protein percent among non-nodulating ( $rj_1$   $rj_1$ )  $F_2$  plants averaged 1.6 times those of their nodulating ( $Rj_1$ ) sibs. Estimates of environmental variance components, however, were made from very small samples of the homozygous non-nodulating parent, noduleless (CO) 'Clark' and the normal nodulating  $P_2$  parents. Normal Clark is (CN). Heritability estimates, therefore, were not as accurate as desirable. A similar study was needed which included more adequate estimates of environmental components of variance from CO and CN or similar lines to be applied to populations of segregating generations.

It would seem appropriate for estimates of variance from the CO parental line to be subtracted from the phenotypic variance of  $F_2$ ,  $rj_1$   $rj_1$  plants to estimate the genetic component of that  $F_2$  sub-population. Similarly estimates of variance obtained from plants of CN could be subtracted from the phenotypic variance of  $F_2$ ,  $Rj_1$   $Rj_1$  plants to estimate the genetic variance component of that  $F_2$  sub-population. In a like manner, variances among hills of CO plants and hills of CN plants could be used as estimates of the non-genetic components of phenotypic variances among  $F_3$  hills of  $rj_1$   $rj_1$  and  $Rj_1$   $Rj_1$  sub-populations respectively.

This report presents seed oil data from parental lines,  $P_1$  (which is CO in our case) and  $P_2$ , the normal counterpart of CO (which is CN), and  $F_2$  and  $F_3$  hybrid generations associated with three soybean crosses. Percent oil was chosen because it can be measured easily and in small seed quantities by nuclear magnetic resonance (NMR). Furthermore, the correlation between percent oil and percent protein is negative but quite high.

All crosses had CO as the female parent. One had 'Mandell', one had 'Wisconsin Black' and one had a Genetic Type Collection line, T245, as the male parent. Mandell has about 19% oil whereas Wisconsin Black and T245 have about 16%.

Plants of parental lines and  $F_2$ 's were grown approximately 30 cm apart in rows approximately 38 cm apart and 5.8 m long. Rows of CO, CN,  $P_2$  and  $F_2$  plants were randomized in blocks, one block for each cross. Seeds were harvested by individual plant and dried to 4% moisture. Oil percentages were estimated by NMR. Sixteen seeds from each plant were inoculated and grown in a mixture of sand and vermiculite for six weeks after which they were examined for the presence of nodules. If all seedlings had nodules their parental  $F_2$  plant was assigned the genotype  $Rj_1$   $Rj_1$ , if none had nodules the  $F_2$  parent was  $rj_1$   $rj_1$  and if some had nodules while others did not the  $F_2$  parent plant was classified as  $Rj_1$   $rj_1$ .

Table 1

Variances in oil percentages among CO, CN, P<sub>2</sub> and F<sub>2</sub> plants  
involved in three soybean crosses (1976)

Cross (P <sub>1</sub> x P <sub>2</sub> )	CO (P <sub>1</sub> )	CN	P <sub>2</sub>	<u>Rj<sub>1</sub>Rj<sub>1</sub></u> (F <sub>2</sub> )	<u>rj<sub>1</sub>rj<sub>1</sub></u> (F <sub>2</sub> )
CO x Mandell	3.85 (40)	1.81 (39)	2.04 (37)	2.56 (47)	3.15 (62)
CO x T245	3.86 (21)	1.80 (20)	5.77 (18)	3.18 (59)	5.26 (55)
CO x Wisconsin Black	2.77 (40)	1.65 (40)	9.62 (35)	3.65 (83)	4.36 (64)

\*Number in sample in parentheses.

Table 2

Variances in seed oil percentages among progeny hills of CO<sub>1</sub>, CN,  
P<sub>2</sub> and F<sub>3</sub>'s involved in three soybean crosses (1977)

Cross (P <sub>1</sub> x P <sub>2</sub> )	CO (P <sub>1</sub> )	CN	P <sub>2</sub>	<u>Rj<sub>1</sub>Rj<sub>1</sub></u> (F <sub>3</sub> )*	<u>rj<sub>1</sub> rj<sub>1</sub></u> (F <sub>3</sub> )
CO x Mandell	0.57 (14)	0.21 (14)	0.51 (14)	1.07 (92)	1.58 (92)
CO x T245	0.58 (14)	0.18 (14)	1.10 (14)	0.76 (87)	0.99 (86)
CO x Wisconsin Black	0.87 (14)	0.14 (14)	0.21 (14)	1.10 (92)	1.22 (87)

\*Degrees of freedom in parentheses, pooled over two replications. In some cases there were missing hills in one or both replications.

Table 3

Heritability estimates (broad sense) for variation in seed oil percentages  
among F<sub>2</sub> plants and F<sub>3</sub> progenies associated with three soybean crosses

Cross	F <sub>2</sub> plants (1976)		F <sub>3</sub> progenies (1977)	
	<u>Rj<sub>1</sub>Rj<sub>1</sub></u>	<u>rj<sub>1</sub>rj<sub>1</sub></u>	<u>Rj<sub>1</sub>Rj<sub>1</sub></u>	<u>rj<sub>1</sub>rj<sub>1</sub></u>
CO x Mandell	0.29	-0.22	0.80	0.64
CO x T245	0.43	0.27	0.76	0.41
CO x Wisconsin Black	0.55	0.36	0.87	0.29



Sample sizes ranged from 20 to 40 within crosses but totaled 99 for CN. Those for CO ranged from 21 to 40 and totaled 101 over all three crosses. Data from these samples were applied to  $F_2$  material. Estimates for the  $F_3$  material came from eight hills of CO plants (16 over two replications) and eight hills of CN (16 over two replications).

$F_3$  progenies consisted of two hills per  $F_2$  family. One hill each of these, plus eight hills of each parental line as well as CN, were planted in each of two replications. Ten seeds were planted per hill. Entries were completely randomized within each replication. Hills were planted 30 cm apart in rows 76 cm apart. Each hill was harvested separately and its seed were sampled for NMR analysis.

Variances in seed oil percentages were higher for  $F_2$   $rj_1$   $rj_1$  plants than for their  $F_2$   $Rj_1$   $Rj_1$  sibs in all three crosses (Table 1) although significantly so only in the cross CO x T245. But variances of CO plants were significantly larger than those of CN plants. As a result the estimated environmental variance components for  $rj_1$   $rj_1$   $F_2$  plants were greater than those for  $Rj_1$   $Rj_1$   $F_2$  plants and estimates of heritabilities (in the broad sense) were lower for the noduleless than for the nodulated  $F_2$  sub-populations (Table 3).

Evidence for genetic variance in the noduleless sub-population, in fact, is questionable because the variances among  $rj_1$   $rj_1$  segregates (Table 1) were not significantly greater than that of the CO parent. Significant components for genetic variances, however, were present in the  $Rj_1$   $Rj_1$   $F_2$  sub-populations in two of the crosses (CO x T245 and CO x Wisconsin Black).

Variances of  $F_3$   $rj_1$   $rj_1$  progeny hills were larger than those of  $F_3$   $Rj_1$   $Rj_1$  progeny hills but significantly so only in cross CO x Mandell (Table 2). Variances of CO hills, however, were significantly greater than those of CN hills. Therefore the environmental component in the  $F_3$  progeny variances should be larger in the  $rj_1$   $rj_1$  than in the  $Rj_1$   $Rj_1$  subgroup. Variances of  $F_3$   $Rj_1$   $Rj_1$  progenies were significantly larger than those of the CN parent indicating a real genetic variance component in the former in each of the three crosses. Only one variance of  $F_3$   $rj_1$   $rj_1$  progenies, however, was larger than that of the CO parent, in the cross CO x Mandell. Apparently no genetic component existed in the  $F_3$   $rj_1$   $rj_1$  progenies of the other two crosses. Estimates of heritabilities of differences among  $F_3$  progeny hills are lower for the  $rj_1$   $rj_1$  portion of the population (Table 3).

We used only the noduleless Clark parent (CO) of the crosses or its normal counterpart (CN) for estimating environmental variance components. We did not use the  $P_2$  parent because in each case it was normal ( $Rj_1$   $Rj_1$ ) for nodulation and would seem inappropriate for use with the noduleless  $rj_1$   $rj_1$  portions of the segregating generations. We did not even use the  $P_2$  parents for estimates to apply to the  $Rj_1$   $Rj_1$  portions because such use would make comparisons between  $rj_1$   $rj_1$  and  $Rj_1$   $Rj_1$  subgroups unfair. If the  $P_2$ 's had been used in crosses CO x T245 and CO x Wisconsin Black estimates of  $h^2$  for  $F_2$ 's would have been quite low because variances of T245 and Wisconsin Black plants were surprisingly high.

The data presented here certainly do not suggest that selection in the noduleless portion of  $F_2$  or  $F_3$  would be more effective than selecting in the nodulated portion. In fact they suggest the opposite. A significant genetic variance component in the  $Rj_1$   $Rj_1$   $F_2$  sub-population indicates there are genetic differences for utilizing combined nitrogen from the soil, fixed

nitrogen from the air or both. The sub-population made up of related rj<sub>1</sub> rj<sub>1</sub> plants should have the same array of genotypes except for genes on the chromosome segment that carries the rj<sub>1</sub> locus. A failure of this portion of the population to express a significant genetic variance suggests that in our material genetic variation exists for the system involved with fixed nitrogen but cannot express itself in the absence of Rj<sub>1</sub>.

#### Reference

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#### 4) Relay cropping of soybeans and oats.

One possibility of increasing land productivity in Illinois is to double crop soybeans following wheat. This practice has been limited to the southern half of the state because of the shorter growing season in the northern half. A modification of double cropping known as relay cropping might allow the earlier establishment of soybeans in wheat or oats and extend the northern limit of double cropping in the state. Considerable work has been reported concerning double cropping, but relatively little has been published regarding relay cropping with soybeans (Brown and Graffis, 1976; Lassiter, 1973).

We have begun a study to determine the responses of 14 soybean cultivars representing Maturity Groups I through IV to relay planting in oats. We hope this study will help to answer the question, "Does the soybean breeder have to look for genotypes that differ from those of current cultivars adapted to monoculture in order to exploit efficiently the relay cropping environmental situation?"

Materials and methods: 'Lang' oats were planted April 14, 1978 in rows 41 cm apart. The unusually late planting was forced upon us by continual rains and the late arrival of spring. All the soybean cultivars (see Tables 1 and 2) were planted on May 27, 1978. The experimental design was a split plot with three replications. Monoculture and interplanting (relay planting) were the main plots and were arranged as randomized complete blocks. Subplots (cultivars) consisted of four rows 3.4 meters long and 41 cm apart. A space of 82 cm was left between adjacent plots.

On July 19, the oats were harvested by combine set to cut a height of 51 cm to obtain a maximum yield of oat grain with a minimum amount of removal of soybean plant tissue. Data were taken from the soybeans for lodging, plant height, and number of branches per plant just prior to harvest. Yield was estimated by harvesting 3 m of the two middle rows of each plot. The beans were harvested as they matured between September 19 and October 16.

Results and discussion: Tables 1 and 2 contain data for the traits measured on the soybean cultivars in relay cropping and in monoculture, respectively. Final values were calculated to determine significant differences and

Table 1  
Values for plant traits of 14 soybean cultivars  
(relay cropped in oats)

Cultivar	Yield (kg/ha)	Lodging	Height (cm)	Branches/plant
Wells	618 a	1.2	49	.14
Corsoy	803 ab	3.0	52	.21
Harcor	918 ab	3.0	56	.58
Hark	1,067 abc	2.3	55	.48
Amsoy 71	1,221 cd	2.7	58	.46
Beeson	1,431 cde	2.6	62	.88
Elf	1,445 cde	3.0	45	.43
Wayne	1,517 de	3.1	72	1.78
Cumberland	1,560 de	2.5	62	1.30
Woodworth	1,645 def	1.9	67	1.75
Union	1,720 ef	3.1	70	2.01
Oakland	1,745 ef	2.9	70	1.66
Cutler 71	1,845 ef	3.7	80	2.07
Williams	2,076 f	2.3	68	1.88
X..	1,403			

Table 2  
Values for plant traits of 14 soybean cultivars (monoculture)

Cultivar	Yield (kg/ha)	Lodging	Height (cm)	Branches/plant
Hark	2,452 a	3.1	88	1.22
Amsoy 71	2,764 ab	3.1	105	2.17
Corsoy	2,788 ab	3.1	105	1.85
Cumberland	2,953 abc	3.3	91	2.70
Woodworth	3,116 abc	3.3	98	2.32
Elf	3,284 abc	1.1	58	2.51
Beeson	3,317 abc	3.2	101	2.13
Williams	3,328 abc	3.1	104	2.08
Cutler 71	3,441 bcd	3.0	129	1.89
Oakland	3,735 bcd	2.1	104	3.36
Harcor	3,772 cd	3.1	109	2.09
Wells	3,794 cd	3.1	105	1.30
Wayne	3,835 cd	3.1	129	1.71
Union	4,372 d	3.0	121	1.20
X..	3,359			

those yields not followed by the same letter are considered significantly different. An analysis of variance showed significant effects of cultivars. The interaction indicates that the cultivars respond differently when grown in different cultural systems. This is very important because if such an interaction holds over more environments it would indicate the need to evaluate cultivars in a relay cropping system before recommendations should be made about which cultivar to use in such a system. Also it would indicate that the breeder must select for performance under these conditions.

The results of the correlations made are given in Table 3. Correlations of special interest are the  $r$  values of yield in oats vs. yield in monoculture, and height vs. yield in oats. The low or perhaps non-existent correlation of yield (in oats) vs. yield (monoculture) was expected because of the previously mentioned interaction of cropping systems and soybean cultivars. The relatively high correlation between height and yield within the relay cropping system probably results from those with later maturity being able to take greater advantage of the remaining growing season after oat harvest, i.e., grow more after oat harvest and thus yield more. There are several possible explanations for the poor yields of several varieties. One was that some of the plots had poor germination because of dry conditions at Urbana that lasted from about April 20 to June 15. Most of the lower yielders were the earlier cultivars that were setting pods at the time of oat harvest. Regrowth from these was minimal. They may have yielded more had they been planted later, at about the time of heading of the oats. Some of the better yielders indicate, however, that a very real potential exists for relay cropping to increase land productivity.

The oat yields (from the relay cropping treatment) averaged about 2,000 kg/ha. If bean yields of 2,000 kg/ha are added to such oat yields the system of relay cropping could be viewed as being profitable--especially if these levels of yields can be proven to be reliable. Further study will be conducted in 1979 with a similar experimental design and at several locations in the state of Illinois.

Table 3  
Correlations of various plant traits of soybeans

Yield (in oats)	vs. Yield (monoculture)	$r = .2138$ N.S.
Lodging (in oats)	vs. Lodging (monoculture)	$r = -.2372$ N.S.
Height (in oats)	vs. Height (monoculture)	$r = .7184^{**}$
Branches/plant (in oats)	vs. Branches/plant (monoculture)	$r = .1564$ N.S.
Lodging (in oats)	vs. Yield (in oats)	$r = .2245$ N.S.
Height (in oats)	vs. Yield (in oats)	$r = .8856^{**}$
Branches/plant (in oats)	vs. Yield (in oats)	$r = .6600^*$

\*Significant at 5% level.

\*\*Significant at 1% level.

N.S. = not significant.



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#### 1) Induced cytoplasmic sterility in soybeans.

One of the M<sub>4</sub> progenies of PK-71-39 soybean irradiated with 10 Kr gamma rays showed segregation for sterility in soybean in 1976. It had 18 sterile plants and 4 normal plants, indicating that a single dominant gene was responsible for sterility. The sterile plants had no seeds and, therefore, this appeared to be a dead end for this mutant. Nevertheless, the 4 normal plants were separately harvested and their progenies evaluated in 1977. The results were very interesting, as indicated in Table 1.

Table 1  
Breeding behavior of normal plants from segregating rows

Progeny no.	No. of plants	
	Sterile	Fertile
1	35	2
2	53	1
3	5	0
4	<u>22</u>	<u>1</u>
Total	115	4

As evident from the table, all the 4 progenies consisted primarily of sterile plants with occasional fertile ones. Pooled over all progenies, there were 115 sterile plants and 4 fertile plants. The progenies of these 4 normal plants were again evaluated in 1978. The results were very similar to what was observed in 1977, as indicated in Table 2.

Table 2  
Breeding behavior of second generation normal  
plants from segregating rows

Progeny no.	No. of plants	
	Sterile	Fertile
1	2	0
2	16	1
3	29	0
4	<u>45</u>	<u>1</u>
Total	92	2

These data indicate a definite pattern. In every generation, the progenies consist of about 97-98% sterile plants and 2-3% normal plants. These normal plants again give rise to similar progenies in the succeeding generation. Apparently, sterility in this line seems to be determined by the cytoplasm. The occasional fertile plants probably arise due to temporary restoration of fertility in the out-crossed seeds produced on the fertile plants in the previous generation. Thus, the external pollen provides a restoration factor whose effect lasts for one generation, as suggested in Table 3.

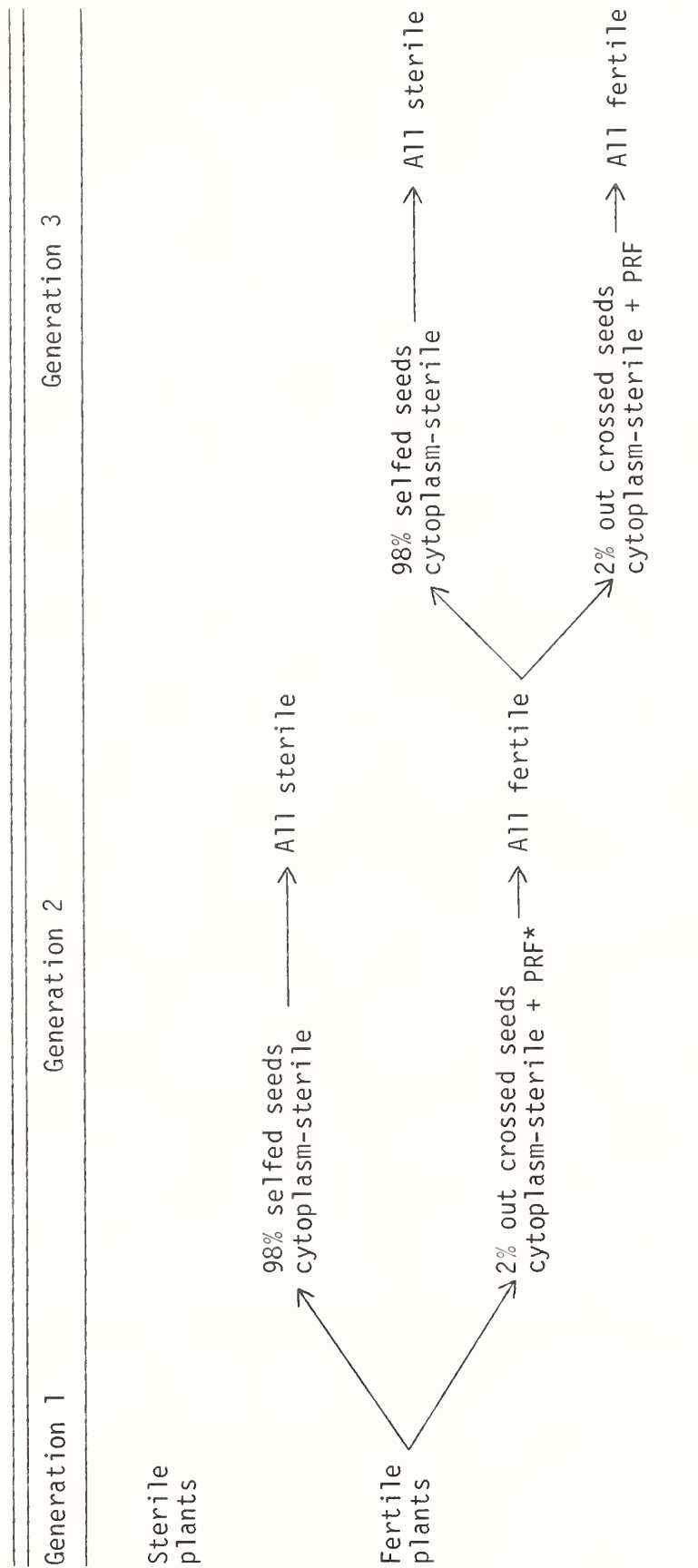
Attempts were made to verify this assumption by artificial pollination on the normal plants in 1978. However, only a few crosses could be attempted because of the limited number of buds on the two normal plants. Consequently no success was achieved.

The sterile plants were indistinguishable from the normal ones until the onset of flowering, after which the differences became apparent. The flowers of sterile plants had small aborted pollen which did not take aceto-carmin stain. In order to check female fertility, about 500 flowers were artificially pollinated with normal pollen but no seed set was observed. Thus, this mutant involved both male and female sterility.

As it is, this mutant has no practical utility and it may probably be lost in the next generation. However, this has indicated a possibility of inducing cytoplasmic male sterility in crop plants.



Table 3  
Possible mechanism for fertility restoration



\*Paternal restoration factor.

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### 1) Evaluation of soybean germplasm.

Soybean has been called the miracle crop of the twentieth century. It is one of the most nutritious among beans and pulses, having 40% protein and 20% oil. In view of the chronic shortage of protein and oil in India, soybean is a welcome introduction to provide the much needed stability and boost to the production of these two essential items of food. Its high nutritive value makes it ideally suited for its versatile industrial uses. Its increasing industrial exploitation has also led to the manufacture of a large number of antibiotics in our country. Well drained upland soils of Chotanagpur have been found to be ideally suited for soybean cultivation.

Improvement work on soybean has been started only recently in the state of Bihar under I.C.A.R. scheme. Information on the various aspects of quantitative characters of germplasm lines are lacking in this crop.

A collection of 261 germplasm lines were obtained from various sources and were sown on 9 July 1977 in single rows. Twenty kg N, 80 kg P<sub>2</sub>O<sub>5</sub> and 40 kg K<sub>2</sub>O per hectare were applied at the time of sowing. The lines were harvested from 1 October to 3 November 1977. All the germplasm lines were studied and were found to breed true. Five plants were selected at random from each row and observations on days to maturity, plant height, 100-seed weight and seed yield/plant were recorded. The mean values for each quantitative character with respect to all the 261 germplasm lines were obtained. The range of variability with respect to 4 quantitative characters are as follows:

S. no.	Characters	Range of variability
1	Days to maturity	85-118
2	Plant height (cm)	15-100
3	100 seed weight (gm)	3.7-19.5
4	Seed yield/plant (gm)	4.1-73.0

The above table indicates a wide range of variability with respect to all the four quantitative characters studied in the 261 germplasm lines of soybean. Promising lines with respect to different quantitative characters are shown in the table on the following page.

This information will be useful for the plant breeders engaged in soybean breeding programs in India.

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Promising lines	
<u>Earliest</u> (85 days)	UPSM-558 and UPSM-665
<u>Latest</u> (118 days)	EC-14459, EC-85609, EC-34354, EC-1555 and EC-18676
<u>Dwarf</u> (15 cm)	UPSM-712 and PK-71-6
<u>Tall</u> (100 cm)	EC-18227, EC-3943, EC-13050 and EC-161171
<u>Bold-seeded</u> (17-19.50 gm per 100 seeds)	Plasso-43, EC-7042, EC-2575, UPSM-167 and UPSM-176
<u>High yielding</u>	EC-15976, IC-15965, EC-9990, EC-3943, EC-13004 and EC-18018

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# 1) Aneuploids and chromosome aberrations from irradiated soybeans.

Irradiation treatment of seeds, pollen, or sporocytes has been used successfully to produce aneuploids in a number of genera. When Dr. E. G. Hammond had finished selecting  $M_1$  plants from his neutron irradiation experiment, I had the opportunity to go through his radiated material to select off-type and semi-sterile plants and to determine the kinds of aneuploids produced by radiation of soybeans.

One- and two-seeded pods from remnant  $M_1$  plants were harvested, the  $M_2$  plants grown in the field, and the  $M_3$  progenies of  $M_2$  plants with more than 20% aborted pollen grains were checked for chromosome number and aberrations, using root tip squashes. The results of the pollen grain and cytological analyses are presented in Tables 1 and 2, respectively.

Table 1  
The number of plants of the  $M_2$  generation with  
20% or more aborted pollen grains

Cultivar	Number of plants		%
	Normal pollen	Aborted pollen	
Amsoy	72	13	15.3
Beeson	55	5	8.3
Corsoy	64	3	4.5
Hark	77	7	8.3
Hodgson	101	25	19.8
Steele	62	11	15.1
Wells	39	2	4.9

Table 2  
The number of  $M_2$  plants yielding diploids, aneuploids,  
or chromosome aberrations in  $M_3$  progenies

Cultivars	Number of $M_2$ plants	Chromosome number			
		39	40	41	42
Amsoy	13	1*	11	2	
Beeson	5		5		
Corsoy	3		2	1	
Hark	7		2 <sup>a</sup>	5	
Hodgson	25		21 <sup>b</sup>	4	
Steele	11		5 <sup>c</sup>	6	2
Wells	2		1 <sup>d</sup>	2	

\*Plant 162-20 yielded 39, 40 and 41 chromosome plants.

<sup>a</sup>Plant 169-14 yielded one plant with 2 short chromosomes and another with 2 long chromosomes.

<sup>b</sup>Plant 171-31 yielded one plant with 2 long chromosomes. Plant 172-11 yielded one plant with 2 short satellite chromosomes and 2 long chromosomes, two plants with 1 long and 1 short satellite chromosomes, and two plants with 1 short satellite chromosome.

<sup>c</sup>Plant 175-7 yielded a 41 chromosome plant and a 40 chromosome plant with 2 short chromosomes.

<sup>d</sup>Plant 178-7 yielded a 41 chromosome plant and a 40 chromosome plant with 1 short chromosome.

Plant 162-20-17, an  $M_3$  progeny with 39 somatic chromosomes, was a thick-stemmed, vigorous plant that was semi-sterile and matured late. The chromosome number of this plant was confirmed by observations of microsporocytes with 19 bivalents and 1 univalent. Ten progenies of this monosomic plant had 40 chromosomes, the diploid number. More progenies will be grown and their chromosome number determined.

Plant 172-11, an  $M_2$  progeny, appears to carry a reciprocal translocation involving the satellite chromosome. Twenty bivalents were observed in 172-11-3, an  $M_3$  progeny with two short satellite chromosomes and two long chromosomes. Four other  $M_3$  progenies of 172-11 had no observable chromosome aberrations, two had one short satellite and one long chromosomes, and two had one short satellite chromosome. Plant 172-11-3 and the four with no observable chromosome aberrations were fertile whereas the remaining four plants with either a short satellite chromosome or a short satellite chromosome and a long chromosome were late maturing and semi-sterile.

The results indicate that irradiation of soybeans may be as good a method for producing aneuploids as screening asynaptic or desynaptic mutants. A monosomic soybean plant found in the  $M_3$  progeny was of particular interest because hypoploids have not been found among aneuploid progenies from asynaptic or desynaptic mutants.

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### 1) Spontaneously occurring sterile plants.

Two sterile plants were found in a commercial field of soybeans in Ames in 1977. The plants were noticed because of their retention of chlorophyll when fertile plants had matured and turned brown. One of the plants, 'Sterile A', had set two one-seeded pods, and the other, 'Sterile B', had set 7 seeds.

Other researchers have mentioned or reported the spontaneous occurrence of sterile plants in commercial fields and, commonly, the apparent lack of a genetic determinant for the sterility. Our analysis of descendants of Steriles A and B indicates that genetic sterility is probably lacking in these two cases, also, and that the general occurrence of sterile plants in commercial fields may be due, in part, to ploidic and/or genomic instability.

Progeny of Steriles A and B had elevated chromosome numbers (Table 1) and were highly sterile, except for one plant, D9. D7 was also sterile, except that one branch set several pods. Whether seed formation on Steriles A and B resulted from self- or cross-pollination is not known, since segregation of genetic markers was unexpected.



Chromosome counts of progeny from D7 and D9 revealed low aneuploid chromosome numbers (Table 2), indicating the loss of extra chromosomes and a regression toward the basic 40-chromosome constitution.

Genetic sterility was not evident among the eight viable progeny of D7 or the 28 viable progeny of D9. Reduced seed-set occurred among plants having 42, 43 and 44 chromosomes, as expected, but the lack of sterility among other plants indicated that a recessive or dominant monogenic sterility system had not brought about reduced seed-set on Steriles A and B. Progeny of one 40-chromosome D7 descendant and four 40-chromosome D9 descendants were screened for segregation. Forty-seven to 50 progeny of each plant failed to segregate sterility.

Table 1  
Chromosome numbers of progeny from Steriles A and B

Sterile A		Sterile B	
Plant number	Chromosome number	Plant number	Chromosome number
D6	70	D8	68
D7*,†	52	D9†	43
		D10	48
		D11	58
		D12	68
		D13	(Died)
		D14	(Died)

\*One axillary branch of D7 was relatively fertile, and set several seeds.

†Progeny of D7 and D9 were analyzed further (see Table 2).

Table 2  
Chromosome numbers of D7 and D9 progeny

Chromosome number	D7 progeny	D9 progeny
40	1*	5*
41	1	13
42	3	6
43	2	3
44	1	1
Unknown	2	2

\*Progeny of the one D7 descendant and four of the five D9 descendants having 40 chromosomes were screened for segregation of sterility genes in the F<sub>3</sub> generation.

We suspect that the sterility of Steriles A and B resulted from highly elevated chromosome numbers, either euploid or aneuploid. Spontaneous triploids and tetraploids can arise from occasional 2N gametes, and the former are likely to produce highly aneuploid progeny. The data are compatible with this hypothesis, and do not indicate a monogenic system of sterility. We also suspect that the failure of other workers in their attempts to isolate genetic sterility systems readily may be explainable on a similar basis, i.e., cases of sterility in commercial fields could result from abnormal numbers of chromosomes. Plant diseases probably also contribute to the number of naturally occurring sterile plants. The selection of green plants bearing few seed at maturity, therefore, need not lead to the isolation of a genetic sterility system.

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## 2) A partially male-sterile mutant in soybeans.

An entry consisting mostly of plants having little to no seed set was found amidst the breeding material of Dr. Walter R. Fehr (Iowa State University) in 1975. The entry was descendent from germplasm population AP6(S1)C1, which was described by Fehr and Ortiz (1975). Investigations have revealed that partial male sterility was the primary cause leading to reduced seed set (Stelly, 1979).

Observations of fresh and paraffin-embedded material manifested the partial male sterility. The ability of partially male-sterile plants to set seed from self-pollination and cross-pollination, and cytological observations, revealed that female fertility is not the factor that limits the amount of seed set by partially male-sterile plants. On the other hand, abnormal female development sometimes occurred, but its incidence was high only among floral buds formed after the regular period of flowering (plants bearing few or no seed continue to flower).

The trait is controlled by a single recessive allele (Table 1). Phenotypic expression of the partial male sterility is highly variable, and subject to modification by background genotype and environment. The amount of selfed seed set on homozygous recessive plants varies considerably, due to incomplete expression of male sterility. When genetically sterile plants set large amounts of seed, they are phenotypically indistinguishable from genetically fertile plants at maturity. Modification of the phenotypes leads to occasional misclassification and, thus, the large homogeneity  $\chi^2$  for families shown in Table 1. This interpretation is favored over the alternative explanation that heterogeneity resulted from digenic epistatic inheritance of the trait; progeny tests of fertile  $F_2$  plants gave results expected under the hypothesis of monogenic control, but not digenic control (Table 2).

The gene pleiotropically affects corolla morphology such that standard petals do not bend back, and instead enclose the wing and keel petals. Expression of this floral trait also is variable. Flowering is prolonged when seed set is low; abnormal floral bud differentiation becomes increasingly

Table 1  
Segregation of msp msp plants

Segregation <sup>a</sup> Fertile : Sterile	Monogenic inheritance			Homogeneity		
	$\chi^2$	d.f.	P	$\chi^2$	d.f.	P
3289 : 1091	0.0195	1	0.95-0.99	6.12 <sup>b</sup> 106.58 <sup>c</sup>	6 93	0.25-0.5 0.075-0.10

<sup>a</sup>Pooled data from segregating  $F_2$  and  $F_3$  families.

<sup>b</sup>Contingency test for homogeneity of populations.

<sup>c</sup>Contingency test for homogeneity of families.

Table 2  
Progeny tests of fertile  $F_2$  plants

Type of $F_3$ family Segregating : Nonsegregating	Chi-squares and probabilities			
	Monogenic <sup>a</sup>	Probability	Digenic <sup>b</sup>	Probability
77 : 39	0.00	1.0-0.9	19.09**	0.00-0.01

<sup>a</sup>If monogenic, the expected ratio of segregating:nonsegregating families from fertile  $F_2$  plants is 2:1.

<sup>b</sup>If digenic with epistasis (i.e., 13:3  $F_2$  ratio), the expected ratio of segregating:nonsegregating families from fertile  $F_2$  plants is 6:7.

\*\*Significant at the 0.01 probability level.

frequent and fleshy pods are produced as sterile plants age. Plant maturation is normal and vestigial pods are not produced when seed set is normal or nearly normal.

The capacity of the partially male-sterile plants to self-fertilize under certain conditions is reflected by the capacity of homozygous recessive plants to set large numbers of seed and pods and by the preponderance of partially male-sterile plants among the progeny of partially male-sterile seed parents. In some cases, sterile plants have produced more than 100 seeds from self-pollination. The ability of partially male-sterile plants to self-pollinate under certain conditions will allow for the synthesis of large, homogeneous populations of genetically sterile plants, as once suggested by Smith (1947). Such populations will be male sterile if grown in an appropriate environment. The proportion of seed that is cross-pollinated seems to vary

inversely with the total amount of seed set on partially male-sterile plants, but controlled experiments to determine the levels of outcrossing have not been conducted to date. A large population of partially male-sterile plants homozygous for w<sub>1</sub> is being generated, however, for this purpose.

This mutant line has been assigned Genetic Type Collection T-number T271H and the gene symbol msp by the Soybean Genetics Committee.

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### 3) A cytologically identifiable short chromosome.

Seeds set on partially male-sterile plants (see article 2 for a description of the sterility system) were grown in the greenhouse in the spring of 1977. One of the plants, designated D56, had an unusual growth habit--the plant was somewhat spindly, climbing, and had a thin main stem. It had been noted at the time of transplanting that the root system of the D56 seedling consisted of a very long tap root and unusually thin lateral roots. Petiole cuttings were made in order to check the chromosome number of D56, but we were unable to establish the chromosome number of the plant. Subsequent pollen sampling revealed semi-sterility among pollen grains (41.7% of the pollen grains were aborted, i.e., they did not stain in I<sub>2</sub>KI). Ovule abortions were frequent also, giving further evidence of gametophyte inviability.

The exact source of the semi-sterility is unknown, since D56 resulted from a natural cross-pollination. But several of our observations indicate that G. soja, "G. gracilis", or an introgression product of one of these species with G. max, was the male parent of D56. First, the growth habit of D56 was more like that of "G. gracilis" than of G. max. Second, D56 was heterozygous for L<sub>1</sub> (black pod) and homozygous for T (tawny pubescence); the partially male-sterile female parent was l<sub>1</sub>l<sub>1</sub>TT, and the only L<sub>1</sub>L<sub>1</sub>TT material grown in the field in 1976 was descendent from Plant Introductions of G. soja and "G. gracilis". Third, seeds formed by D56 were somewhat small, their seed coats were an off-yellow color (perhaps an indirect effect of L<sub>1</sub>) and their dark hila were uniformly ringed by a narrow region of the seed coat that was pigmented; this sort of seed pigmentation normally is not observed in G. max x G. max crosses. Fourth, segregation of F<sub>2</sub> genotypes led to an array of seed coat colors on F<sub>3</sub> seed (maternal tissue), including the dark, speckled seed coat found in G. soja and "G. gracilis". Thus, we are reasonably confident



that the semi-sterility, and the short chromosome described below, came from one of these species or from an introgression product.

Root tips from seeds produced by D56, later generations and testcrosses have been used to determine chromosome numbers. Analysis of D56 progeny revealed numerous cases of aneuploidy. Just as important, the presence of an abnormally short chromosome was noted. The small chromosome is roughly one-half of the size of the smallest chromosome in the *Glycine max* complement; it is slightly sub-metacentric. The chromosome is readily identifiable in well-spread mitotic metaphases.

In addition to identifying a variety of aneuploid conditions (Table 1) that involve only the short chromosome, we have found several aneuploid plants whose aneuploidy involved one or more univalent shifts. Rate of transmission of the small chromosome has been high among self-progeny, and moderately so in cross-pollinations. Our data concerning transmission of the larger trisomic chromosomes (from univalent shifts) are presently too limited to allow an inference on the rate(s) of transmission for that/those chromosome(s).

Table 1

Types of chromosome constitutions that occurred among the progeny of D56

Chromosome number	Type of extra chromosome			
	None	One short	Two short	One normal*
40	+	+	- <sup>a</sup>	-
41		+	- <sup>b</sup>	+
42		+	+	+

\*'Normal', referring to a chromosome that was not short.

<sup>a</sup>We have screened a few progeny from plants having 39 normal and one short chromosomes, but have not recovered plants with 38 normal and two short chromosomes.

<sup>b</sup>We have found plants with 39 normal and two short chromosomes among progeny from plants having 39 normal and one short chromosome.

Preliminary analysis of meiosis has indicated that the small chromosome is often present as a univalent at metaphase 1 in PMC's, and as cytoplasmic bodies in tetrads. Quantitative data have not been collected yet. We have not observed configurations suggesting the presence of a translocation, deletion, or inversion, to date, though either of the first two types of aberrations might have been involved in the formation of the small chromosome. Certain features of the distributions of pollen and ovule abortions across karyotypes suggest that such an aberration may be segregating in the material.

Our work presently involves determining rates of transmission for the small chromosome and its derivatives resultant from univalent shifts, testing for homology among the new aneuploids, and between the new aneuploid(s) and



Trisomics A, B and C. Studies of the meiotic behavior of the chromosomes will be included.

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#### 4) Seed coats of *Glycine soja* and "*G. gracilis*"--inheritance of color/pattern.

In the preceding note, it was mentioned that the derivation of an abnormally short chromosome involved natural cross-pollination of a partially male-sterile (msp msp) plant (A76-517-2) by pollen from G. soja, "G. gracilis", or an introgression product of these species into G. max. The recessive allele for self seed coat color (i) and the allele(s) producing the dark seed coat pattern of G. soja and "G. gracilis" were concomitantly transferred in the cross-pollination. Segregation in later generations and a few testcrosses indicate that the characteristically patterned seed coats of G. soja and "G. gracilis" are governed by an allele of the R locus; the allele appears to be dominant to r (brown), r<sup>m</sup> (ring-pattern) and, perhaps, to R (black). For the purpose of this note, however, we will refer to the patterned seed coat as the 'soja-type'.

We have observed segregation of the soja-type seed coat in families descendent from parents having the soja-type or yellow seed coats, but not in families descendent from plants having brown seed coats. This led us to believe that the patterned seed coats of G. soja and "G. gracilis" might be dependent on the presence of an r allele. Limited data from F<sub>2</sub> plant segregation are compatible with this hypothesis (Table 1).

Table 1

Segregation of plants having either soja-type or brown seed coats

Generation	Segregation		d.f.	$\chi^2$	Probability
	<u>soja-type</u> :	brown			
<u>F<sub>2</sub></u>	23 :	8	1	0.0107	
<u>F<sub>2</sub></u>	21 :	6	1	0.1111	
Sum	44 :	14	1	0.0229	0.9-0.95

One hybrid plant was produced from a cross-pollination of an i i r<sup>m</sup> r<sup>m</sup> (T125) plant with pollen from a plant having the soja-type seed coat. The hybrid produced F<sub>2</sub> seed having the soja-type seed coat (maternal tissue), indicating that the soja-type allele is dominant over r<sup>m</sup>. F<sub>2</sub> plants will be grown in the summer of 1979, and their seed classified for seed coat color/pattern.

Expression of the soja-type seed coat is dependent on the lack of I. All  $F_1$  plants from crosses between plants having the soja-type seed coat color and those homozygous for I produced seeds with yellow or green seed coats. In two crosses between the soja-type and plants of the I I T T W<sub>1</sub> W<sub>1</sub> R R genotype (yellow seed coat and gray hilum),  $F_1$  plants produced seeds with yellow seed coats and dark hila.

Further information on the allelism and order of dominance for the soja-type seed coat will be obtained as additional testcross and segregation data are collected.

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#### 5) A new chlorophyll mutant.

A new recessive chlorophyll mutant was unexpectedly recovered in an  $F_2$  family of A76-518-3 (a homozygous partially male-sterile, msp msp, plant) x A76-669 (a 'Clark' isoline homozygous for the chromosome translocation from PI 101,404B). Furthermore, the translocation did not appear in the  $F_2$  generation. We are uncertain as to whether the intended cross was unsuccessful and followed by a natural outcross, or if a new chromosomal rearrangement had taken place. The former seems more likely. The  $F_2$  population segregated the partial male sterility trait, so we are certain that a cross was involved.

Field-grown plants homozygous for the mutant allele first manifest abnormal chlorophyll content as seedlings; progressive chlorosis and necrosis sometimes kills seedlings, but others survive as short spindly plants. This mutant differs from T265H in that yellow plants often survive both in the field and greenhouse. Shading from healthy (green) sibs seems to promote the health of the chlorotic seedlings and plants. Seedlings which survive and flower often set a few seed. We have noted that the amount of shading given to plants in the greenhouse also affects the longevity of the mutants. Temperature, too, may influence viability of the plants; one mutant plant that was grown in a shaded region of a cool greenhouse remained relatively healthy and produced a large number of seed.

A further indication that environment affects expression of the allele comes from the observation of seedlings grown in a greenhouse sandbench. Initial screening of  $F_3$  families was done in the greenhouse during the winter of 1977-1978. Five seeds from each of 48  $F_3$  families were sown and grown to the three-trifoliate leaf stage. Chlorosis was not observed among any of the families grown in the sandbench, but was observed among field-grown sibs. Low light intensity in the greenhouse during the winter months may have precluded expression of the chlorosis.

Data from  $F_2$  segregation of the new mutant are compatible with the hypothesis of monogenic recessive control of the mutant phenotype (Table 1).  $F_3$  data, however, are only marginally compatible with the same hypothesis, due to a relative deficiency of mutant phenotypes (Table 1). Although  $F_3$  families were homogeneous for their ratios of segregation, the overall deficiency of mutant phenotypes warranted tests for the possibility of digenic epistatic inheritance.

Table 1  
Segregation data for a chlorophyll-deficient phenotype

Generation (year)	Segregation		Monogenic recessive		Homogeneity		
	Normal : Yellow		$\chi^2$	Probability	d.f.	$\chi^2$	Probability
F <sub>2</sub> 1977	92	34	0.265	0.75-0.50	-	-	-
F <sub>3</sub> <sup>a</sup> 1978	1118	327	4.322*	0.05-0.025	36	39.75	0.5-0.25

<sup>a</sup>Data from segregating families only.

\*Significant at the 0.05 probability level.

F<sub>3</sub> families from F<sub>2</sub> green plants included 37 segregating families and 11 nonsegregating (all green) families. These results are compatible with the hypothesis of monogenic recessive inheritance for the yellow phenotype, but are incompatible with the hypothesis of digenic epistatic control (Table 2). We conclude, therefore, that a single mutant recessive allele controls the chlorotic phenotype. The mutant has been assigned soybean genetic type collection T-number (T270H), but has not been assigned a gene symbol, due to the possibility of allelism with previously designated alleles that are maintained at Urbana, Illinois.

Table 2  
F<sub>3</sub> analysis of green F<sub>2</sub> plants

Generation	Segregating : Nonsegregating	Chi-squares and probabilities			
		Monogenic <sup>a</sup>	P	Digenic <sup>b</sup>	P
F <sub>3</sub> families	37 : 11	2.344	0.25-0.10	18.487**	0.01-0.00

<sup>a</sup>If monogenic, the expected ratio of segregating : nonsegregating families from green F<sub>2</sub> plants is 2 : 1.

<sup>b</sup>If digenic with epistasis (i.e., 13 : 3 F<sub>2</sub> ratio), the expected ratio of segregating : nonsegregating families from green F<sub>2</sub> plants is 6 : 7.

\*\*Significant at the 0.01 probability level.

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6) Inheritance and expression of a mutant phenotype affecting the number of petals per flower.

Plants of the Glycine max Plant Introduction 68,704 characteristically produce flowers that have six or more petals, rather than the normal complement of five petals (1 standard, 2 wing, and 2 keel petals). We have investigated the inheritance and expression of this trait.

Eight  $F_1$  plants were classified by sampling ten flowers per plant; none of the  $F_1$  plants produced more than five petals, indicating that the phenotype is under recessive genetic control. Since all of the crosses employed PI 68,704 as female and L15 as male, we cannot eliminate the possibility of a cytoplasmic interaction with nuclear control.

Data from  $F_2$  segregation indicate that the trait is controlled digenically and that plants homozygous for recessive alleles at either locus can produce flowers having more than five petals (Table 1).

The production of extra petals by mutant plants is a variably expressed trait; normal plants produce extra petals only very rarely. In plants of this Plant Introduction, every flower seems to be affected, albeit variably. A sampling of 10 flowers from each of 9 plants yielded no instance where only five petals were present. Extra wing and keel petals occurred more frequently than did extra standard petals (Table 2). In contrast, the level of expression was much more erratic among flowers of mutant plants that segregated in the  $F_2$  families; many flowers contained only the normal complement of petals. The distribution of extra petals among the different petal types seems to have been altered; also the number of extra wing and standard petals were similarly low, but the number of extra keel petals remained relatively high (Table 2).

Whether or not incomplete epistasis accounts for all or part of the differences observed between plants of this Plant Introduction and  $F_2$  families can be tested through statistical analysis of expression on mutant plants having known genotypes. Such plants will become available as backcrosses and testcrosses are made.

Table 1  
Data from  $F_2$  plant segregation of normal and mutant plants,  
from the cross PI 68,704 x L15

	Segregation		$\chi^2$ d.f.	Probability
	Normal	Mutant		
Observed	83 : 56			
Exp. (3:1) <sup>a</sup>	104.25	34.75	17.326	0.0-0.005
Exp. (9:7) <sup>b</sup>	78.19	60.81	0.677	0.25-0.50

<sup>a</sup>Segregation ratio expected under monogenic recessive control.

<sup>b</sup>Segregation ratio expected under digenic recessive control, where the recessive condition at either locus is epistatic to dominant alleles at the other locus.

Table 2

Mean number of extra petals per flower, by types of petals,  
for parental, F<sub>1</sub> and F<sub>2</sub> plants

Line	Petal types			Average
	Keel	Wing	Standard	
PI 68,704	0.933 (0.067) <sup>a</sup>	0.755 (0.073)	0.300 (0.053)	0.663 (0.041)
L15	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
F <sub>1</sub>	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
F <sub>2</sub> normals	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
F <sub>2</sub> mutants	0.350 (0.0296)	0.082 (0.015)	0.041 (0.084)	0.158 (0.012)

<sup>a</sup>Standard errors of means are given parenthetically.

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#### 7) Reference diagrams of seed coat colors and patterns for use as genetic markers in crosses.

Specht and Williams (1978) reported on the use of hilum color as a genetic marker in soybean crosses. We present the classification of seed coat color and seed coat patterns that we have been using. Table 1 lists the genes affecting seed coat pigmentation to be considered. Table 2 presents data from the 64 genotypic combinations according to flower and pubescence color. Table 3 summarizes the data.

Table 1

Genes affecting seed coat pigmentation

Gene	Phenotype	Gene	Phenotype
I	light hilum	R	black seed
i	dark hilum	r	brown seed
ik	saddle	T	tawny (brown) pubescence
i	self dark color	t	gray pubescence
O	brown seed	W <sub>1</sub>	purple flower
o	reddish brown seed	w <sub>1</sub>	white flower



Table 2  
Genotypic combinations for seed coat, saddle and hilum colors

TW and Tw*					tw					tw				
R0    Ro    r0    ro					R0    Ro    r0    ro					R0    Ro    r0    ro				
I	G**	G	Y	Y	I	G	G	Y	Y	I	Y	Y	Y	Y
i	B1	B1	Br	Rbr	i	Ib	Ib	Bf	Bf	i	Bf	Bf	Bf	Bf
ik	B1	B1	Br	Rbr	ik	Ib	Ib	Bf	Bf	ik	Bf	Bf	Bf	Bf
i	B1	B1	Br	Rbr	i	Ib	Ib	Bf	Bf	i	Bf	Bf	Bf	Bf

\*See Table 1 for complete description of T, t, W, w, R, r, O, o, I, ii, ik and i.

\*\*G = gray, B1 = black, Br = brown, Rbr = reddish brown, Y = yellow, Ib = imperfect black, Bf = buff. Seed coat color is yellow or nearly so in I and ii genotypes and matches the hilum color in i genotypes. Saddle color (ik genotypes) also matches the hilum color.

Table 3  
Summary of 64 genotypic combinations for seed coat, saddle and hilum colors

Genes		Phenotypes			
		Self color	Saddle & hilum color	Hilum color	Hilum color
		i	ik	ii	I
T	R	black	black	black	gray
T	r	0	brown	brown	yellow
T	r	o	reddish brown	reddish brown	yellow
t	R	W <sub>1</sub>	imperfect black	imperfect black	gray
t	R	w <sub>1</sub>	buff	buff	yellow
t	r		buff	buff	yellow

The use of genetic markers for distinguishing between hybrid and 'self' progeny is even more important when making cross-pollinations (Walker *et al.*, 1979). As Specht and Williams (1978) have pointed out, hilum and seed coat colors may be used as genetic markers when flower, pubescence and pod color are not useful markers.

Seed coat and hilum phenotypes corresponding to combinations of alleles at five gene loci (I, R, O, T and W) are presented in this report. The O locus was not considered by Specht and Williams, but it, too, can be employed

as a genetic marker for checking cross-pollination success. The O and I gene loci are linked, with  $17.8 \pm 0.7\%$  recombination (Weiss, 1970); segregation at O and I loci may generate unexpected phenotypes in certain crosses.

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#### 8) A flower structure mutant.

A flower structure mutant was found segregating within the original heterogeneous PI 339,868 population in 1970. This mutant is characterized by having cleistogamous flowers with an exposed stigma and is sterile.

The flowers of sterile plants have been observed by dissections, serial paraffin sections and the scanning electron microscope (SEM). Flower development and structure were found to be abnormal. The petals of these flowers grow abnormally and eventually surround the stamens. Consequently, staminal tube elongation is blocked. At anthesis the anthers are positioned around the ovary rather than around the stigma, and the petals are curved over the top of the anthers. Self-pollination is prevented by the spatial separation between the anthers and stigma and by the physical barriers of the petals.

The actual cause of sterility in this mutant has not been determined. It is not male sterile. Pollen grains produced by sterile plants stain normally with  $I_2KI$  and frequently have been observed germinating in vivo with the SEM and in paraffin serial sections.

This mutant may have some degree of female sterility. Megasporogenesis, observed in paraffin serial sections, looks normal. However, in 200 hand pollinations attempted, using the sterile plant as female, only 1 seed was produced. This lack of crossing success could be due to the absence of normal indicators of female receptiveness (petal size and color) or it could be due to the exposed stigma drying out before the female is receptive. On the other hand, the lack of crossing success using the mutant as female could be a result of female sterility.

The fact that self pollination does not take place has been documented by dissections, serial paraffin sections and SEM. This, by itself, could lead to sterility in this mutant. However, further study is needed to determine the degree of female sterility and the contribution it makes to sterility in this mutant.

Segregation for fertility: sterility within PI 339,868 is 3:1 (Table 1). However, when this parent population is crossed to genetically unrelated populations, the resulting  $F_2$  ratio in segregating families is 15 fertile plants to every sterile plant (Table 2). These ratios indicate that the sterility is controlled by two genes and that both genes must be homozygous recessive to produce a sterile plant. From the data in Tables 1 and 2, we conclude that PI 339,868 is homozygous recessive for one gene and segregating for the second gene.

This sterile was tested for linkage with several other traits. To date, no linkage has been detected (Table 3). Other linkage tests are in progress.

This mutant has been designated  $fs_1fs_1fs_2fs_2$  (flower structure) and has been given Genetic Type Collection Number T269 by the Soybean Genetics Committee. Thus, the original PI 339,868 population is considered to be  $Fs_1fs_1fs_2fs_2$  and will be maintained as T269H.

Table 1  
Segregation within PI 339,868

Year	Family			$\chi^2(2:1)$	P	Total	Plant		$\chi^2(3:1)$	P
	Total	Seg.	Not seg.				Fertile	Sterile		
1971	12	8	4	0.000	1.00	176	136	40	0.485	<.50
1972	84	60	24	0.857	<.50	2048	1532	516	0.417	<.75
1974	86	57	29	0.006	<.96	2168	1637	531	0.298	<.75

Table 2  
 $F_2$  segregation in crosses with PI 339,868

Populations crossed to	Total plants	Segregation		$\chi^2(15:1)$	P
		Fertile	Sterile		
Hark	1189	1105	84	1.348	<.25
Clark	437	408	29	0.112	<.75
Clark T/T	717	670	47	0.114	<.75
T93	813	752	61	2.176	<.25
T219H	361	336	25	0.282	<.75
T230	211	198	13	0.003	<.975
T241	2555	2396	159	0.003	<.975
T242	1974	1845	129	0.273	<.75
T258	1201	1114	87	2.026	<.25

Table 3  
Linkage tests with PI 339,868

Trait tested	Segregation				Expected ratio	$\chi^2$	P
	Fertile		Sterile				
	W	w	W	w			
Flower color	1023	332	76	30	45:15:3:1	3.41	<.50
Pubescence color	T	t	T	t	45:15:3:1	3.39	<.50
	827	283	60	26			
Clark translocation							
Normal/50% aborted	Normal	50%	Normal	50%	15:15:1:1	3.087	<.50
	349	321	19	28			
Trisomic C							
40 chromosomes	480		36		15:1	0.465	<.50
41 chromosomes	625		48		15:1	0.895	<.50
T241 ( <u>st</u> <sub>2</sub> ) <sup>†</sup>	2424		652		51:13	1.485	<.25
T242 ( <u>st</u> <sub>3</sub> ) <sup>†</sup>	1244		316		51:13	0.003	<.975
T258 ( <u>st</u> <sub>4</sub> ) <sup>†</sup>	2177		530		51:13	0.900	<.50

<sup>†</sup>In F<sub>2</sub> populations segregating both loci from PI 339,868 and st<sub>2</sub>, st<sub>3</sub> or st<sub>4</sub>, we expect a 153:19:16:4 ratio. Since all sterile genotypes have identical phenotypes at maturity, all sterile plants are grouped, producing a 153:39 ratio (simplified to 51:13).

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#### 9) Genetics of the meiotic mutant st<sub>5</sub>.

In 1970, a part-sterile plant in Uniform Test I, entry W6-4108 (from Wisconsin), was observed at Ames, Iowa. Seven seeds from this part-sterile plant gave rise to seven plants in 1971; six were fertile and one was sterile and set no seeds. In 1972, five plant progeny rows gave all fertile plants, i.e., they did not segregate fertile and sterile plants. Plant progeny row A72-441-3 segregated 30 fertile:13 sterile plants. Twenty-two plant progeny rows were planted in 1973; 14 segregated both fertile and sterile plants and 8 had only fertile plants. Table 1 summarizes the frequencies of fertile and sterile plants in segregating F<sub>2</sub> families. The 122 sterile plants set no seed.

Pollen grains from the Wisconsin sterile were stained with I<sub>2</sub>KI. Pollen grains were small, shrunken and collapsed, and were similar in appearance to pollen grains from st<sub>2</sub>, st<sub>3</sub> and st<sub>4</sub> plants. Microspore mother cells of sterile plants were examined, and a low level of chromosome pairing was observed, indicating that the sterile was either an asynaptic or desynaptic mutant.

Three nonallelic asynaptic or desynaptic mutants have been reported previously in soybeans. Hadley and Starnes (1964) reported st<sub>2</sub> (T241) and st<sub>3</sub> (T242) and Palmer (1974) described st<sub>4</sub> (T258). Winger et al. (1977) described a spontaneous mutant at the st<sub>2</sub> locus.

The purpose of this study was to determine if this new asynaptic or desynaptic mutant, the Wisconsin sterile, st<sub>?</sub>, is allelic to either st<sub>2</sub>, st<sub>3</sub> or st<sub>4</sub>. This was accomplished by crossing known heterozygotes, i.e., St<sub>2</sub>st<sub>2</sub> x St<sub>?</sub>st<sub>?</sub>, St<sub>3</sub>st<sub>3</sub> x St<sub>?</sub>st<sub>?</sub> and St<sub>4</sub>st<sub>4</sub> x St<sub>?</sub>st<sub>?</sub>. F<sub>1</sub> and F<sub>2</sub> populations of each cross were observed. If two lines were allelic with regard to their sterility, then one out of four F<sub>1</sub> plants would be sterile; in the F<sub>2</sub> generation, non-segregating families and families segregating 3 fertile:1 sterile plants would be observed. If different genes were controlling sterility in the two lines, however, no sterile plants would be observed in the F<sub>1</sub> generation. Moreover, the F<sub>2</sub> generation would include nonsegregating families, families segregating 3 fertile:1 sterile plants, and families segregating 9 fertile:7 sterile plants.

No sterile plants were found among F<sub>1</sub> plants from the three genetic combinations of T241H, T242H and T258H with the Wisconsin sterile, respectively. Among segregating F<sub>2</sub> populations, two groups were evident on the basis of the Chi-square values (Tables 1, 2, 3 and 4). One group seemed to represent a 3:1 population; the other group seemed to represent a 9:7 population. These results agree with the hypothesis that the recessive gene in the Wisconsin sterile is different from the genes in T241, T242 or T258. As a result of the present study, this mutant was assigned a Genetic Type Collection T-number (T272) and the gene symbol st<sub>5</sub> by the Soybean Genetics Committee. This line is maintained as the heterozygote, T272H.

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Table 1

Frequencies of fertile and sterile plants in segregating F<sub>2</sub> families of the Wisconsin sterile (W6-4108)

Year	Fertile	Sterile	$\chi^2(3:1)$	P
1973	377	122	0.08	<0.90



Table 2

Ratio of fertile to sterile plants in segregating<sup>a</sup> F<sub>2</sub> families from crosses between heterozygous plants of T258 and heterozygous plants of the Wisconsin sterile

	Fertile plants	Sterile plants	$\chi^2$ (3:1)	P	Fertile plants	Sterile plants	$\chi^2$ (9:7)	P
Totals	588	196	5.24	<0.90	80	59	0.18	<0.75
Pooled $\chi^2$			0.00	0			0.10	<0.90
Homogeneity $\chi^2$			5.24	<0.90			0.08	<0.90

<sup>a</sup>10 families appeared to segregate 3:1 and 2 families 9:7.

Table 3

Ratio of fertile to sterile plants in segregating<sup>a</sup> F<sub>2</sub> families from crosses between heterozygous plants of T242 and heterozygous plants of the Wisconsin sterile

	Fertile plants	Sterile plants	$\chi^2$ (3:1)	P	Fertile plants	Sterile plants	$\chi^2$ (9:7)	P
Totals	1138	385	9.56	<0.99	459	336	3.05	<0.975
Pooled $\chi^2$			0.06	<0.90			0.71	<0.50
Homogeneity $\chi^2$			9.50	<0.975			2.34	<0.975

<sup>a</sup>20 families appeared to segregate 3:1 and 9 families 9:7.

Table 4

Ratio of fertile to sterile plants in segregating<sup>a</sup> F<sub>2</sub> families from crosses between heterozygous plants of T241 and heterozygous plants of the Wisconsin sterile

	Fertile plants	Sterile plants	$\chi^2$ (3:1)	P	Fertile plants	Sterile plants	$\chi^2$ (9:7)	P
Totals	1169	362	15.02	<0.90	215	147	3.52	<0.75
Pooled $\chi^2$			1.50	<0.25			1.45	<0.25
Homogeneity $\chi^2$			13.52	<0.95			2.07	<0.75

<sup>a</sup> 23 families appeared to segregate 3:1 and 5 families 9:7.

Reid G. Palmer—USDA

10) Inheritance of male-sterile, female-fertile mutant  $ms_3$ .

Two non-allelic male-sterile strains each controlled by a single recessive gene,  $ms_1$  (Brim and Young, 1971) and  $ms_2$  (Bernard and Cremeens, 1975), respectively, have been reported in soybeans. We now have evidence for a third completely male-sterile type controlled by a single recessive gene at a different locus from either  $ms_1$  or  $ms_2$ . As a result of the present study, this mutant was assigned a Genetic Type Collection T-number (T273) and the gene symbol  $ms_3$  by the Soybean Genetics Committee. This line is maintained as the heterozygote T273H.

In 1971, in an  $F_3$ -derived line from the cross 'Calland' x 'Cutler', Dr. John Thorne of Northrup, King & Co., Washington, Iowa, observed several sparsely podded plants. Fertile plants in this plant progeny row were harvested and evaluated in 1972. In segregating families, we found approximately 3 fertile:1 sterile plants (529:183, expected 534:178).

Sterile plants had normal-appearing anthers but pollen grains were poorly stained with  $I_2KI$  and were slightly smaller than pollen grains from fertile plants. Microspore mother cells of sterile plants were examined; meiosis was normal. As soon as the microspores were released from the tetrad, however, they began to abort. Pollinations were made on sterile plants with a success rate nearly as high as on fertile plants (51% pod set versus 56% pod set, respectively).

In order to test the relationship of the Northrup, King male-sterile to  $ms_1$  and  $ms_2$ , we made crosses using male-steriles as the female parent and heterozygotes as the male parent (Tables 1 and 2). All  $F_1$  plants were fertile. In the  $F_2$ , as would be expected if  $ms_1$  or  $ms_2$  and the Northrup, King male sterile were at separate and unlinked loci, half of the families segregated 3:1 and half segregated 9:7 (Tables 1 and 2).

The inability to identify male-sterile plants before flowering severely restricts use of this mutant in commercial hybrid seed production, but this mutant may be useful in genetic or plant breeding experiments.

Table 1  
Male-sterile allelism tests between  $ms_1ms_1$   
and Northrup, King male sterile

	$ms_1ms_1$ x T273H							
	3:1 segregation				9:7 segregation			
	Total fertile	Total sterile	d.f.	$\chi^2$	Total fertile	Total sterile	d.f.	$\chi^2$
Totals	1033	351	7	2.02	899	699	6	1.51
Pooled $\chi^2$ (1 d.f.)			1	0.10			1	0.00
Homogeneity $\chi^2$			6	1.92			5	1.51

Table 2  
Male-sterile allelism tests between  $ms_2ms_2$   
and Northrup, King male sterile

	$ms_2ms_2$ x T273H							
	3:1 segregation				9:7 segregation			
	Total fertile	Total sterile	d.f.	$\chi^2$	Total fertile	Total sterile	d.f.	$\chi^2$
Totals	505	168	10	2.29	315	251	6	6.68
Pooled $\chi^2$ (1 d.f.)			1	0.01			1	0.08
Homogeneity $\chi^2$			9	2.28			5	6.60

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### 11) Inheritance of male-sterile, female-fertile mutant $ms_4$ .

The previous article mentioned male-sterile mutants  $ms_1$  and  $ms_2$  and described the genetics of a new male-sterile mutant,  $ms_3$ . We now have evidence for a fourth completely male-sterile type controlled by a single recessive gene at a different locus from either  $ms_1$ ,  $ms_2$  or  $ms_3$ . As a result of the present study, this mutant was assigned a Genetic Type Collection T-number (T274) and the gene symbol  $ms_4$  by the Soybean Genetics Committee. This line is maintained as the heterozygote T274H.

In 1973, one sparsely podded plant was observed in a field of 'Rampage' grown at the Bruner Farm near Ames, Iowa. All three progeny from this plant were grown in the greenhouse and were fertile. Plant progeny rows were grown in 1974. One plant row was completely fertile, one plant row segregated both fertiles and steriles and tawny and grey pubescence; one plant row segregated fertiles and steriles and was similar in appearance to Rampage. This last plant row, A74-4646-2, is the source of the Rampage male sterile.

Fertile plants in plant progeny row A74-4646-2 were harvested and evaluated in 1975. In segregating families we found approximately 3 fertile:1 sterile plants (640:208, expected 636:212). Sterile plants had normal-appearing anthers but pollen grains clumped and stained poorly with  $I_2KI$ . Microspore mother cells of sterile plants were not examined. Pollinations were made on sterile plants with a success rate as high as on fertile plants (52% pod set versus 49% pod set, respectively).

In order to test the relationship of the Rampage male sterile to  $ms_1$ ,  $ms_2$  and  $ms_3$ , we made crosses using male steriles as the female parent and heterozygotes as the male parent (Tables 1, 2 and 3). All  $F_1$  plants were fertile. In the  $F_2$ , as would be expected if  $ms_1$  or  $ms_2$  or  $ms_3$  and the Rampage male sterile were at separate and unlinked loci, half of the families segregated 3:1 and half segregated 9:7 (Tables 1, 2 and 3).

As is the situation with  $ms_1$ ,  $ms_2$  and  $ms_3$ , the inability to identify male-sterile plants before flowering severely restricts use of these mutants in commercial hybrid seed production.

Table 1  
Male-sterile allelism tests between Rampage  
male sterile and  $Ms_1ms_1$

	T274 x $Ms_1ms_1$							
	3:1 segregation				9:7 segregation			
	Total fertile	Total sterile	d.f.	$\chi^2$	Total fertile	Total sterile	d.f.	$\chi^2$
Totals	755	242	7	3.56	453	327	6	3.55
Pooled $\chi^2$ (1 d.f.)			1	0.91			1	1.06
Homogeneity $\chi^2$			8	2.65			7	2.49

Table 2  
Male-sterile allelism tests between  $ms_2ms_2$   
and Rampage male sterile

	$ms_2ms_2$ x T274H							
	3:1 segregation				9:7 segregation			
	Total fertile	Total sterile	d.f.	$\chi^2$	Total fertile	Total sterile	d.f.	$\chi^2$
Totals	930	303	8	2.42	873	723	6	8.79
Pooled $\chi^2$			1	0.12			1	1.56
Homogeneity $\chi^2$			9	2.30			7	7.23



Table 3  
Male-sterile allelism tests between Rampage  
male sterile and  $Ms_3ms_3$

	T274 x $Ms_3ms_3$							
	3:1 segregation				9:7 segregation			
	Total fertile	Total sterile	d.f.	$\chi^2$	Total fertile	Total sterile	d.f.	$\chi^2$
Totals	1326	421	13	8.40	984	786	8	3.43
Pooled $\chi^2$			1	0.76			1	0.31
Homogeneity $\chi^2$			14	7.64			9	3.12

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#### 1) Inheritance of resistance to necrotic strain of SMV in soybean.

A necrotic strain of soybean mosaic virus (SMV) is one of the most destructive diseases in some leading soybean cultivars of Korea and its infection sometimes causes complete loss of the crop. The necrotic disease reported first as a strain of soybean mosaic virus in 1976, affects the most promising commercial cultivars, 'Kwangkyo' and 'Gangrim', which have been cultivated extensively since released in 1969. Hence, an investigation on the mode of inheritance of resistance gene in soybean cultivars was undertaken to develop resistant lines to the necrotic virus disease by mutation technique, which is being carried out with the cultivar Kwangkyo at present.

Paschal and Goodman (1978) reported resistance to a severe isolate of soybean mosaic virus in cultivar 'Buffalo' to be conditioned by one or more dominant genes. Three resistant soybean cultivars and a Korean native line were engaged to cross with the susceptible cultivar Kwangkyo. The  $F_1$  plants for each of the four crosses were grown in the field, and flower, pubescence and seed coat colors were used as genetic markers to verify the hybridization. Both  $F_1$ ,  $F_2$  plants and parents were grown in the field and inoculated with extract of infected leaves by conventional rubbing method at 2-4 leaf stage, being put aphids to enhance natural infection, too.

The  $F_1$  hybrids of each cross between Kwangkyo and #31926, KEX-2, 'Kumgang-daerip', KAS 390-10 were susceptible, indicating that resistance is controlled by recessive gene (Table 1). In determination of disease reactions of the  $F_2$  populations, it was segregated in a ratio of 3 susceptible to 1 resistant, thus confirming that resistance is conditioned by a single recessive gene. For further evidence, backcrosses and  $F_3$  generations are to be

Table 1

Segregation of infection types of  $F_1$ ,  $F_2$  and parents inoculated with necrotic strain of soybean mosaic virus

Exp. no.	Cross	Generation	Number of plants			$\chi^2$ value	P value (3:1)
			Resistant	Susceptible	Total		
I	Kwangkyo(S) <sup>a</sup> x #31926(R)	P <sub>1</sub>		28	28		
		P <sub>2</sub>	30		30		
		F <sub>1</sub>		10	10		
		F <sub>2</sub>	52	128	180	1.45	0.10-0.25
II	Kwangkyo(S) x KEX-2(R)	P <sub>1</sub>		30	30		
		P <sub>2</sub>	30		30		
		F <sub>1</sub>		6	6		
		F <sub>2</sub>	6	16	22	0.06	0.75-0.90
III	Kwangkyo(S) x Kumgang-daerip(R)	P <sub>1</sub>	1	29	30		
		P <sub>2</sub>	30		30		
		F <sub>1</sub>		6	6		
		F <sub>2</sub>	3	15	18	0.66	0.25-0.50
IV	Kwangkyo(S) x KAS390-10(R)	P <sub>1</sub>		30	30		
		P <sub>2</sub>	28		28		
		F <sub>1</sub>		8	8		
		F <sub>2</sub>	6	20	26	0.04	0.50-0.75

<sup>a</sup>Disease reaction; R = resistant and S = susceptible.

tested. From the results, it is expected that resistant mutants induced from the cultivar Kwangkyo by irradiation will be selected in a few generations without drastic changes of other agronomic characters of the mother variety.

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### 2) Preliminary studies for screening techniques on shade tolerance of soybean.

Soybean intercropping with other crops usually causes poor yield, mainly by light reduction. Sometimes, a continuous rainfall during the growing season in the area of monsoon is a major factor for yield reduction in soybean by insufficient sunlight as well as shading by intercropping.

Recently our laboratory has collected over 1500 lines as germplasm for Korean native soybean lines and has conducted tests for evaluation of various agronomic characters. With this work, we are interested in selecting the genetic physiological lines adaptable to inadequate growth conditions. Hence, the objective in this study was to determine the effects of light reduction on several agronomic characters to establish an effective screening technique for shading tolerance.

From our germplasm, 16 collected lines having differences in plant type, number of nodes and several growth habits were used for the experiment. Shade treatment was established for 15 days by covering with reeds at a height of 120 cm on the plants from east to west direction and a total of 5 treatments at various growth stages was made during the period 6 July to 18 September 1978. Light reduction in covered plots was estimated at around 56% as compared with control plots.

Response to shade treatment was significantly different among the engaged soybean lines. In general, overgrowth of plant height, reduced number of branch and seed size appeared in second shading period, whereas number of nodes was not affected by shading. Number of pods and seed yield per plant were significantly decreased in all the shading treatments from late flowering to pod filling stages. Consequently, it could be suggested that shading treatment during the pod filling stage would be most effective.

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### 1) Soybean plant design for closed ecological life support system.\*

Prior to the establishment of the space habitats of the future, the life science program office of the National Aeronautics and Space Administration (NASA) is interested in the development of a ground-based manned demonstration of the closed ecological life support system (CELSS). Since CELSS concept centers around complete recycling of all available resources, a genetic plant design to render the total plant more useful is very important. Previous studies (Phillips, 1977; Phillips *et al.*, 1978) conducted for NASA clearly indicate the usefulness of soybean plants in such a system. It has been suggested that 43% of the cropped area in the manufacturing facility in space be planted under soybeans for feed and food in the space habitat (Phillips, 1977). Research on screening and selection of early maturing and high yielding soybean cultivars has also been recommended (Phillips *et al.*, 1978). We feel that besides being early maturing and high yielding, soybean plant should have high seed yield efficiency (SYE). SYE can be defined as the ratio of seed to non-seed dry matter weight. Highly efficient plants, out of the total energy required, will utilize relatively more energy for the production of seed and less for non-seed plant parts. It is possible to select soybean cultivars

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with high seed yield efficiency along with early maturity and high yielding ability (Joshi and Smith, 1976).

The objective of this study is to identify early maturing genotypes with high seed yield efficiency.

Materials and methods: Thirty-one soybean cultivars were planted in the field on June 27, 1978. Fifteen seeds of each cultivar were planted in rows, distance between rows being 91 cm and seed to seed distance being 3 cm, in three replications. At maturity when almost all the leaves had fallen and 95% of pods had turned brown, 5 plants of each cultivar from each replication were harvested. Plants were harvested by hand at ground level and each plant was stored in a cloth bag for further analysis. Above ground biomass at maturity (leaves and roots excluded) was partitioned into three components, i.e., stem and branches, pods, and seeds. These components were dried in an air convection oven at 80°C for 24 hr. After 24 hr drying, the samples were transferred into the dessicator before the actual weighing. The dry matter weight of each component was recorded and the seed yield efficiency of each plant was calculated ( $SYE = \text{seed dry matter wt.} / \text{non-seed dry matter wt.}$ ). Data were analyzed employing ANOVA; Duncan's Multiple Range Test was used to test significant differences between the means.

Experimental results: Five cultivars, PI 196,530, PI 194,640, PI 194,641, PI 189,868 and PI 205,090, matured in the shortest time period and took only 71 days from seeding to maturity. Another three cultivars, 'Maple Presto', 'Sioux' and FC 30,687, took 2 more days to mature (73 days) (Table 1). Among the 31 cultivars tested, 8 took the longest time to mature, i.e., 93 days. Early maturing soybean cultivars are considered a good candidate for the CELSS program. Eight early maturing cultivars which matured in 71-73 days should be examined critically under controlled environments where these should be grown hydroponically.

Among the early maturing cultivars (71-73 days maturity), the highest SYE was obtained from Maple Presto (0.939), followed closely by PI 196,530 (0.934) and Sioux (0.927) respectively (Table 1). However, the variation in SYE among these three cultivars was not significantly different from each other. Other five early maturing cultivars (PI 194,640, PI 194,641, FC 30,687, PI 189,868 and PI 265,090) had significantly lower SYE as compared to Maple Presto and PI 196,530. Though cultivar Sioux gave quite high SYE (0.927), the SYE was not significantly different as compared to PI 194,640 with an SYE of 0.853. The lowest SYE was obtained from PI 205,090 (0.658).

Further studies of Maple Presto, PI 196,530 and Sioux, to determine the total biomass under controlled environment conditions and their compatibility with other food plants, are in progress.

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Table 1  
Seed yield efficiency of certain soybean cultivars

Cultivar	SYE	Seeding to maturity (days)	Cultivar	SYE	Seeding to maturity (days)
PI 196,491	1.223a	93	PI 257,429	0.803e-i	77
PI 194,639	1.158a	93	PI 189,963	0.789f-j	86
PI 196,485	1.001b	93	Ottawa	0.788f-j	77
PI 196,501	0.971bc	86	PI 153,293	0.784g-j	85
Maple Presto	0.939bc	73	PI 194,656	0.783g-j	93
PI 196,530	0.934bc	71	PI 194,641	0.782g-j	71
Sioux	0.927b-d	73	PI 153,296	0.778g-j	85
PI 052,903	0.891c-e	77	PI 159,764	0.773g-j	86
PI 189,867	0.891c-e	86	FC 30,687	0.729h-k	73
PI 196,529	0.858d-f	93	PI 189,868	0.722h-k	71
PI 194,640	0.853d-g	71	PI 196,526	0.715i-k	86
Agate	0.847e-g	77	PI 194,632	0.714i-k	93
PI 154,198	0.836e-g	85	PI 189,869	0.703k	93
Pando	0.831e-g	77	PI 194,633	0.702k	86
PI 196,528	0.823e-g	93	PI 205,090	0.658k	71
PI 196,502	0.814e-h	86			

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## 2) Effect of row spacing and seed rate on soybean pod damage by *Heliothis zea*-Boddie under normal and late planting.\*

Corn earworm (*Heliothis zea*-Boddie) is one of the most destructive pests of soybeans (*Glycine max* [L.] Merrill). Cultural practices, since early days, have been known to play an important role in controlling insect pests in various crops. Some researchers have observed that soybeans with closed canopy escape corn earworm damage (Dietz *et al.*, 1976) but recent reports from extension entomologists in Maryland are contrary to this effect. Since open or close canopy is a function of seed rate and row spacing, the present investigation was undertaken to determine the effect of row spacing and seed rate on soybean pod damage by corn earworm under normal and late planting.

Materials and methods: Soybean cultivar 'Delmar' was planted at normal (May 13) and late (June 24 and July 8) planting times during 1977. Four row spacings, i.e., 11, 23, 46 and 91 cm apart rows, and 3 different seed rates, i.e., 4, 8 and 12 seeds/0.3 m were evaluated. The experiment was laid out in a split plot design with 4 replications. Each plot consisted of 4 rows, each being 6 m long. Net experimental row was 4.9 m long. At maturity, damaged pods were counted on each plant in one of the center rows in each treatment. The results were analyzed statistically using ANOVA and Duncan's Multiple Range Test. Means not followed by the same letter in all tables given in text were statistically different at the 0.05 probability level according to Duncan's Multiple Range Test.

Experimental results: Variations in canopy development were achieved by using different seed rates and row spacings. The number of plants at maturity were not the same as the number of seeds planted/0.3 m for 8 and 12 seeds treatments. The final stand for 8 and 12 seeds was 7 and 9 plants/0.3 m respectively. There was a considerable loss of plants in 12 seeds/0.3 m treatment and this may be attributed to higher competition.

The number of damaged pods for each planting date has been given in Table 1. Though minimum pod damage was observed in June 24 planting, it was not significantly different from May 13 planting. Maximum pod damage was observed in July 8 planting but was not significantly different from May 13 planting. July 8 planting becomes relatively more susceptible to this pest as is indicated by the highest number of damaged pods (Table 1).

Row spacing in soybeans also seems to exert considerable influence on the pod damage (Table 2). Minimum pod damage was observed in rows 11 cm apart. However, this pod damage was not significantly different from that of 46 cm apart rows. Twenty-three and 91 cm row spacing produced the same number of damaged pods and there was no significant difference between these two row spacings and 46 cm apart rows. These data indicate that soybeans planted in 11 cm apart rows, which is virtually a solid stand situation, are not preferred by corn earworm. However, it may be noted that pod damage calculations based on per unit area will yield quite different results. For example, in an area of 4.5 m<sup>2</sup>, 8 rows 11 cm apart, 4 rows 23 cm apart, 2 rows 46 cm apart and only 1 row 91 cm can be accommodated. Pod damage/4.5 m<sup>2</sup> area for various row spacings is given in Table 2. Pod damage/unit area increased significantly as the distance between rows is reduced. This may be due to the fact that more

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Table 1

Effect of planting dates  
on pod damage

Planting date	Damaged pods/ 4.9 m row (#)
May 13	25.4ab
June 24	23.0b
July 8	27.8a

Table 2

Effect of row spacing on pod damage

Row spacing (cm)	Damaged pods/ 4.9 m row (#)	Damaged pods/ 4.5 m <sup>2</sup>
11	22.1b	176.7a
23	27.1a	108.4b
46	25.1ab	50.2c
91	27.2a	27.2d

plants are available for oviposition in narrow row spacings than in wider row spacings.

Pod damage is also influenced significantly by seed rate (Table 3). Maximum pod damage was observed when 8 seeds/0.3 m were planted and the damage was significantly higher than 4 seeds/0.3 m. Though pod damage was higher for 8 seeds/0.3 m than 12 seeds/0.3 m, it was not statistically different from each other. It appears that 4 seeds/0.3 m (4 plants at maturity) and 12 seeds/0.3 m (9 plants at maturity) are not conducive to corn earworm egg laying. This implies that very low and high plant populations are not preferred by corn earworm.

Table 3

Effect of seed rate on pod damage

Seed rate/0.3 m	Damaged pods/4.9 m row (#)
4	23.7b
8	27.3a
12	25.2ab

When soybeans were planted on May 13, minimum pod damage was observed in 91 cm row spacing with 12 seeds/0.3 m and the damage was significantly low as compared with 46 cm row spacing with the seeding rate of 8 seeds/0.3 m and 23 cm row spacing with 4 seeds/0.3 m (Table 4). In the June 24 planting, best results were obtained in 46 cm apart rows with 4 and 8 seeds/0.3 m but the pod damage was not significantly different from the other treatments except when soybeans were planted at the seeding rate of 8 seeds/0.3 m in 11 cm rows in which case the pod damage was maximum. Minimum pod damage was observed in July 8 planting in 11 cm apart rows with 4 seeds/0.3 m. This damage was significantly low as compared with 8 seeds/0.3 m in 23, 46 and 91 cm apart rows, and 12 seeds/0.3 m in 23, 46 and 91 cm apart rows. Maximum pod damage was

Table 4

Pod damage as affected by planting dates x seed rates x row spacing

Planting dates								
May 13			June 24			July 8		
Seed rate/ 0.3 m	Row spacing (cm)	Pod damage/ 4.9 m row	Seed rate/ 0.3 m	Row spacing (cm)	Pod damage/ 4.9 m row	Seed rate/ 0.3 m	Row spacing (cm)	Pod damage/ 4.9 m row
4	11	22.5c-f	4	11	20.8c-f	4	11	13.3ef
4	23	31.3a-e	4	23	27.5a-f	4	23	24.0b-f
4	46	27.0b-f	4	46	18.3d-f	4	46	24.8b-f
4	91	20.8c-f	4	91	26.8b-f	4	91	27.3a-f
8	11	28.3a-e	8	11	31.5a-e	8	11	20.0d-f
8	23	24.3b-f	8	23	25.0b-f	8	23	34.8a-c
8	46	26.3b-f	8	46	18.0ef	8	46	28.0a-e
8	91	28.8a-e	8	91	25.8b-f	8	91	37.0ab
12	11	21.5c-f	12	11	21.8c-f	12	11	19.3d-f
12	23	24.3b-f	12	23	20.5c-f	12	23	32.5a-d
12	46	31.8a-e	12	46	20.5c-f	12	46	31.5a-e
12	91	18.3d-f	12	91	19.5d-f	12	91	41.0a

observed on soybeans planted at the rate of 12 seeds/0.3 m in 91 cm apart rows. These data indicate that pod damage by corn earworm can be reduced by choosing proper seed rate and row spacing for different planting times.

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### 3) Evaluation of soybean germplasm for resistance to corn earworm—III.\*

During previous years (1974-75), soybean cultivars belonging to Maturity Groups 00 to IV were tested in the screenhouse for corn earworm (*Heliothis zea*-Boddie) resistance and the results were reported in the 1978 issue of Soybean Genetics Newsletter (Joshi, 1978a, 1978b). A new batch of soybean cultivars, 30 belonging to Maturity Group IV and 39 to Maturity Group V, were tested in the screenhouse (54' x 72' x 15') during 1976. Ten seeds of each cultivar were planted on June 16, 1976 in 4 replications, the seeds being 2" apart within the row and rows being 36" apart. Screenhouse was infested by releasing 528 corn earworm moths. The moth releases were started on August 16 and continued until August 23. Plants were harvested at maturity and the number of undamaged and damaged pods was recorded for each cultivar. Data were analyzed employing ANOVA; Duncan's Multiple Range Test was used to test significant difference between the means.

The mean numbers of undamaged and damaged pods per plant for each cultivar are reported below. The means not followed by the same letter are significantly different at the 0.05 probability level according to Duncan's Multiple Range Test. Among the 30 cultivars tested in Maturity Group IV, PI 253,652 produced the highest number of undamaged pods/plant (Table 1), followed by cultivar 'Scott' which produced 81.1 undamaged pods/plant. The number of damaged pods/plant for PI 253,652 and Scott were 3.6 and 2.9 respectively. The correlation coefficient between undamaged pods/plant and seed yield was quite high ( $r=0.802$ ). Seed yield/plant was 26.08g and 16.28g for PI 253,652 and Scott respectively. Cultivar 'Scioto' was observed to have the highest number of damaged pods/plant (18.4 pods). Though Scioto showed a high degree of preference for pod damage by corn earworm, its yield (22.45g) was not significantly different from PI 253,652, indicating a high degree of tolerance to this pest.

Among the tested cultivars in Maturity Group V, the highest number of undamaged pods/plant was produced by PI 60,273 (93.4 pods), followed by cultivar 'Peking' (77.5), PI 381,671 (71.3), 'Hill' (68.5), FC 31,721 (65.3),

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Table 1

Mean undamaged and damaged pods for certain soybean cultivars

	Undamaged pods/plant	Damaged pods/plant	Cultivar	Undamaged pods/plant	Damaged pods/plant
<u>Maturity Group IV</u>					
PI 60,970	22.0f	1.43d-f	Mokapu Summer	45.0c-f	5.4b-f
PI 72,227	24.2f	0.68ef	PI 340,010	48.3c-f	2.8b-f
PI 229,319	29.8ef	2.4b-f	PI 226,591	48.6c-f	1.6d-f
PI 61,944	34.8d-f	0.4f	Roe	48.9c-f	8.4b
Bonus	36.2d-f	2.0b-f	PI 253,651 <sup>B</sup>	50.8c-f	2.9b-f
PI 54,617	37.2d-f	5.2b-f	Clark 63	51.1c-f	6.9b-f
PI 246,367	38.3c-f	2.6b-f	Kaikoo	52.7c-f	8.2bc
SRF 450	39.9c-f	3.7b-f	PI 181,550	58.5c-f	2.2b-f
PI 87,623	40.1c-f	1.1d-f	Delmar	60.6c-f	7.3b-d
PI 88,302	41.6c-f	3.8b-f	PI 157,419	63.7b-f	1.4d-f
PI 157,437	42.9c-f	2.1b-f	PI 157,452	68.2b-e	1.6d-f
Cutler 71	43.6c-f	7.1b-e	Bethel	70.4b-e	5.6b-f
PI 340,012	44.1c-f	1.1d-f	Scioto	77.4b-d	18.4a
PI 88,814	44.4c-f	1.8c-f	Scott	81.1bc	2.9b-f
SRF 425	44.9c-f	2.1b-f	PI 253,652	102.7a	3.6b-f
<u>Maturity Group V</u>					
PI 157,470	21.6i	0.4e	FC 31,683	52.0b-i	3.6a-e
PI 157,394	24.0hi	1.7de	PI 71,465	52.1b-i	3.5a-e
PI 83,942	28.5g-i	0.1e	PI 200,450	52.7b-i	2.3c-e
PI 340,051	30.3f-i	1.3de	PI 79,932	53.5b-i	2.2de
PI 81,780S	30.9f-i	2.9b-e	Arlington	54.2b-h	3.1a-e
S-100	33.65e-i	3.7a-e	PI 96,789	54.3b-h	7.2ab
PI 181,546	34.6e-i	2.5c-e	D67,3297	57.9b-g	2.2de
PI 82,589	36.0d-i	2.9b-e	PI 196,177	58.7b-g	1.8de
PI 95,959	36.1d-i	0.7de	Essex	59.2b-g	4.7a-e
PI 340,019	36.4d-i	4.9a-e	Dorman	59.7b-g	5.0a-e
PI 157,451	38.2d-i	4.0a-e	Shore	60.9b-g	2.0de
PI 170,893	39.9c-i	7.8a	PI 342,003	62.4b-f	2.1de
PI 371,611	40.9c-i	4.6a-e	FC 30,265	64.0a-e	5.6a-d
PI 181,544	42.8c-i	3.1a-e	PI 381,675	64.2a-e	3.6a-e
PI 87,542	43.7c-i	1.1de	FC 31,721	65.3a-e	7.1a-c
PI 62,203	45.0c-i	7.3a	Hill	68.5a-d	3.8a-e
PI 65,342	45.7b-i	1.8de	PI 381,671	71.3a-c	4.0a-e
Dortchsoy	49.5b-i	2.1de	Peking	77.5ab	1.4de
York	50.0b-i	3.6a-e	PI 60,273	93.4a	3.4a-e
PI 371,610	51.8b-i	4.4a-e			

PI 381,675 (64.2) and FC 30,265 (64.0). These seven cultivars produced significantly higher number of undamaged pods/plant as compared to the other 32 cultivars tested. Though the highest seed yield/plant (19.2g) was obtained from PI 342,003, this PI produced significantly fewer undamaged pods/plant (62.4). It appears that PI 342,003 might have an excellent ability for compensation. The second high yielding cultivar was PI 60,273 (18.2g) which also happened to be the cultivar with the highest number of undamaged pods/plant.

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### 4) Soybean germplasm resistant to Heliothis zea-Boddie.\*

Corn earworm (Heliothis zea-Boddie) is a very destructive pest of soybeans (Glycine max [L.] Merrill). It feeds both on foliage as well as developing pods. Each larva is capable of damaging 6 to 8.2 pods or 7.1 seeds between 4th and 6th (both inclusive) instars (Boldt et al., 1975; Smith and Bass, 1972). On the Eastern Shore of Maryland, after about the middle of August, when the corn silks have withered and turned brown, corn earworm adults prefer to lay eggs on soybean plants. Other researchers have also found that soybeans become primary host as corn and cotton become more mature (Freeman et al., 1967). Though leaf feeding resistance to corn earworm has been discovered in some soybean cultivars (Hatchett et al., 1976; Joshi and Wutoh, 1972), very little research work has been done to identify soybean germplasm which is capable of resisting pod damage from this pest. The present investigation was undertaken to identify soybean germplasm resistant to pod damage by corn earworm.

Materials and methods: Soybean germplasm (3,045 cultivars) belonging to Maturity Groups 00 to V was evaluated in the field for pod damage by corn earworm from 1974 to 1978. Every year cultivars with pod damage were eliminated from further testing. During 1974, 25 seeds of each cultivar were planted in the field from May 15 to May 28, in rows 91 cm long and 91 cm apart. In 1975, 798 cultivars were evaluated; 550 cultivars from Maturity Groups 00 to IV were planted on May 28 and another batch of 248 cultivars from Maturity Group V was planted on June 5. During 1976, 10 seeds

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of each of 32 cultivars of Maturity Group V and 26 of Maturity Group IV were planted in the field on June 26 in 4 replications. Again in 1977, 478 cultivars were evaluated under late planting conditions and the plantings were made on July 5, 6 and 7 in three replications. Any cultivar with pod damage in any replication was eliminated from further testing. Fourteen seeds of each of the 27 cultivars were planted again on July 1, 1978 in 4 replications. Corn earworm population in the environments for the months of August and September was measured by using blacklight trap.

Experimental results: Corn earworm population for the months of August and September during the selection process is given below.

<u>Year</u>	<u>Total moths</u>
1974	679
1975	968
1976	4,778
1977	6,404
1978	2,591

Corn earworm population in the environment increased markedly every year until 1977 and during 1978 population decreased sharply yet it was a considerably higher level than 1974 and 1975. Maximal severity of infestation occurred during 1977.

During the first year 625 cultivars out of 2,797 did not show any pod damage. Maturity Group V germplasm (248 cultivars) was not included in 1974 test. In 1975, 550 out of 625 cultivars with yellow seed coat were selected for further evaluation and 248 additional cultivars of Maturity Group V were also evaluated. Four hundred and sixty-one out of 550 and 65 out of 248 of Maturity Group V were not damaged by corn earworm. During 1976, out of 32 cultivars of Maturity Group V, five cultivars, namely 'Arlington', 'Peking', PI 96,786, PI 340,051 and PI 371,610, did not show any pod damage; and 11 cultivars (SRF 425, 'Bonus', 'Clark 63', 'Cutler 71', PI 61,944, PI 72,227, PI 87,623, PI 88,304, PI 253,651, PI 253,652 and PI 340,012) of Maturity Group IV did not show any pod damage.

Since it has been discovered that late planted soybeans become more susceptible to corn earworm damage (Dietz *et al.*, 1976; Joshi, 1977), during 1977, 478 cultivars belonging to Maturity Groups 00 through V were again evaluated under late planting conditions. The corn earworm population (6,404 moths) was extremely high during this year and only 27 cultivars escaped damage. These 27 cultivars were again evaluated under late sown conditions in the field during 1978 and none of these cultivars showed any pod damage at maturity. It appears that these cultivars have the capability to resist pod damage. The list of 27 resistant cultivars is given below.

<u>Maturity Group 00</u>	<u>Maturity Group I</u>	<u>Maturity Group II</u>	<u>Maturity Group III</u>	<u>Maturity Group IV</u>	<u>Maturity Group V</u>
Ada	PI 68,572	PI 68,694	PI 70,199	PI 72,227	Arlington
Portage	PI 84,964	PI 68,521	PI 70,500	PI 87,623	Peking
PI 361,108	PI 88,443	PI 68,658	PI 88,354	PI 89,010	PI 96,786
		PI 68,670-2	PI 196,156	PI 229,319	PI 340,051
		PI 70,077			PI 371,610
<u>Maturity Group 0</u>		PI 70,503			
PI 370,057A					
PI 372,424					

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### 1) Characterization of several abnormal nodulation reactions in soybeans.

Several abnormal nodulation reactions in soybeans are known. These range from a complete lack of nodules, caused by the non-nodulating gene (Williams and Lynch, 1954) to plants with normal-appearing nodules (Vest et al., 1973), but low nitrogen fixation as exemplified by the 'Peking'-strain 123 combination. The purpose of the study reported here was threefold. First, we wished to examine several known abnormal nodulation reactions; second, we wished to make comparisons between abnormal and normal nodulation reactions; and third, we wished to evaluate a recently observed abnormal nodulation reaction between Rhizobium japonicum strain 62 and the soybean variety 'Amsoy 71'.

Varieties used in the study were Amsoy 71, 'Anoka', 'Dunfield', 'Hardee' and Peking. Surface-sterilized seed from each variety was inoculated with R. japonicum strains 61, 62, 110, 123 and 138. An uninoculated control for each variety was also included. Leonard jar assemblies were used to maintain sterile conditions. Data were taken on plant height, chlorosis, top dry weight, vegetative stage, nodule number and nodule weight. Total nodule activity (TNA) and specific nodule activity (SNA) were calculated on the basis



of acetylene reduction. The data were analyzed as a set of 25 treatments with three replicates of each treatment in a randomized complete block design.

Noninoculated controls were extremely chlorotic in all cases; however, in 2 of 15 control plots there were a few nodules. Serotyping of these nodules showed them to contain serogroup 123. Since these nodules were few and small, and acetylene reduction measurements showed little reduction of acetylene to ethylene, it was assumed the plants were contaminated late in the experiment. A sample of nodules from each variety-strain combination was also serotyped and some nodules from one replicate of the Dunfield-strain 61 combination were found to contain serogroup 123.

Analysis of variance revealed significant differences ( $p = .01$ ) for all characters measured. Significant differences ( $p = .01$ ) also existed among Rhizobium strains for all characters except TNA and a significant ( $p = .01$ ) strain x variety interaction for all characters except SNA.

On the basis of chlorosis score (Table 1) the 25 strain-variety combinations were divided into two groups. Nineteen combinations had scores of 1.3 or less and were termed normal, while 6 had scores of 3.7 or greater and were termed abnormal. Of the remaining traits examined, only dry weight had the same grouping as chlorosis score. For the traits plant height, nodule weight, vegetative stage, and TNA, one abnormal combination fell into the normal group. Grouping of the combinations for SNA and nodule number showed no relationship to the normal-abnormal grouping for chlorosis.

Examination of the root systems of the abnormal types showed variation in the type of nodulation. The Amsoy 71-strain 61 combination had low total nodule mass. Most nodules were small, but some nodules were large in size. The Dunfield-strain 61 combination was similar to the Amsoy 71-strain 61 combination; however, plants had a somewhat higher nodule number, nodule weight,

Table 1  
Average chlorosis score for 25 strain-variety combinations

Variety	Strain				
	61	62	110	123	138
Amsoy 71	4.7a <sup>†¶</sup>	4.0ab <sup>¶</sup>	1.0c <sup>‡</sup>	1.0c	1.0c
Anoka	1.0c <sup>¶</sup>	1.0c	1.0c	1.0c	1.0c
Dunfield	3.7b <sup>¶</sup>	4.0ab <sup>¶</sup>	1.0c	1.0c	1.0c <sup>§</sup>
Hardee	1.0c	1.0c	1.0c	1.0c <sup>¶</sup>	1.0c
Peking	4.0ab <sup>¶</sup>	1.3c	1.0c	3.7b <sup>¶</sup>	1.0c

<sup>†</sup> Numbers with the same letter do not differ significantly at the 5% level according to Duncan's multiple range test. Adjustments for unequal replication made according to Kramer.

<sup>‡, §</sup> Means were calculated on the basis of two and one values, respectively.

<sup>¶</sup> These combinations are classified as abnormal. All other combinations are classified as normal.



and were slightly less chlorotic. Dunfield and Amsoy 71 in combination with strain 62 resulted in nodules variable in size, ranging from very small and white up to large normal-appearing nodules. Proportionately, more nodules were of normal size with strain 62 than with strain 61. The Peking-strain 61 combination resulted in few nodules, but these nodules were all large. In contrast, the Peking-strain 123 combination had the largest number of nodules of any abnormal combination. The nodules were uniform in size and scattered over the entire root system.

Paired t-tests were also run between members of the abnormal groups. Significant differences ( $p = .05$ ) between the Amsoy 71-strain 61 combination and the Amsoy 71-strain 62 combination existed for plant height, chlorosis score, vegetative stage, nodule weight and SNA. The Dunfield-strain 61 combination as compared with the Dunfield-strain 62 combination differed significantly ( $p = .05$ ) only for vegetative stage. This may have been due to strain 123 contamination in one jar of the Dunfield-strain 61 combination as mentioned previously. Peking with strain 61 differed significantly ( $p = .01$ ) from the Peking-strain 123 combination for only nodule number and nodule weight.

It is interesting to note that in all of the abnormal combinations some nitrogen fixation was occurring. TNA ranged from a low of  $.60 \mu\text{moles C}_2\text{H}_4/\text{jar/hr}$  with the Amsoy 71-strain 61 combination to  $6.58 \mu\text{moles/jar/hr}$  with the Dunfield-strain 62 combination. SNA ranged from  $.5 \mu\text{moles/jar/hr/gm nodule weight}$  for the Peking-strain 123 combination to  $5.49 \mu\text{moles/jar/hr/gm nodule weight}$  for the Amsoy 71-strain 62 combination.

Genetic control of the abnormal nodulation reaction of strains 61 and 62 is apparently conditioned by two different loci. Evidence for this arises in the combination involving Peking and the two strains. With the other four soybean genotypes both strains reacted similarly for chlorosis. Peking in combination with strain 61 resulted in plants which were chlorotic, while the Peking-strain 62 combination was not chlorotic.

It is evident that large amounts of variation existed in the *Rhizobium*-soybean symbiosis. Ordering the combinations for each character showed a 2-fold range for plant height, a 7-fold range for dry weight, a 10-fold range for nodule number, a 20-fold range for nodule weight, a 30-fold range for TNA, and a 14-fold range for SNA. Variability of this type is obviously significant and should be kept in mind if increases in nitrogen fixation are one objective in a soybean breeding project.

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2) Inheritance of abnormal nodulation between *Rhizobium japonicum* strain 62 and the soybean variety Amsoy 71.

To date, four genes are known that result in abnormal nodulation in soybeans. The gene *rj*<sub>1</sub> (Williams and Lynch, 1954; Caldwell, 1966) prevents nodulation with almost all *Rhizobium japonicum* strains. The genes *Rj*<sub>2</sub> (Caldwell, 1966) in combination with strains b7 and b14 of the 3-24-44 serogroup and b122 of the 122 serogroup, *Rj*<sub>3</sub> (Vest, 1970) in combination with strain 33, and *Rj*<sub>4</sub> (Vest and Caldwell, 1972) in combination with strain 61 all result in chlorotic plants with abnormal nodulation. A recent observation at Minnesota revealed that the variety 'Amsoy 71' in combination with USDA *R. japonicum* strain 62 resulted in chlorotic plants. On the basis of this observation experiments were conducted to determine the inheritance of this abnormal reaction.

Crosses were made between Amsoy 71 and 'Anoka' (normal with strain 62). Seed of parents, *F*<sub>1</sub>'s, *F*<sub>2</sub>'s and *F*<sub>3</sub>'s were surface sterilized, inoculated with strain 62, and planted in Leonard jar assemblies. Plants were scored on a scale of 1 (normal green) to 5 (highly chlorotic). All Anoka plants had scores of 1; scores of Amsoy 71 plants ranged from 3 to 5. Of four *F*<sub>1</sub> plants, three had scores of 1 and one had a score of 2. A one-gene model with normal green dominant was hypothesized. Accordingly, three *F*<sub>2</sub> populations were classified and fitted to a 3:1 ratio. Plants with scores of 1 and 2 were considered normal and those with scores of 3 to 5 were considered abnormal. None of the three *F*<sub>2</sub> populations gave a good fit to a 3:1 ratio. They were then fitted to a two-gene (9:7) model (Table 1). Populations B and C gave a good fit, but population A did not. In the experiment for evaluating population A, parental Anoka plants all had scores of 1 and Amsoy 71 had scores of 4 and 5. Many *F*<sub>2</sub> plants, however, had scores of 2 and 3. It seemed likely that some misclassification of this group may have occurred. When this group was arbitrarily divided equally between the "normal" and "abnormal" classes, the fit to the 9:7 ratio was good.

Table 1

Distribution of chlorosis scores within *F*<sub>2</sub> populations from three *F*<sub>1</sub> plants and  $\chi^2$  calculations for a two-gene model

F <sub>2</sub> population	Chlorosis score					χ <sup>2</sup> (9:7)	Prob- ability
	Normal		Abnormal				
	1	2	3	4	5		
A	30	10	36	17	5	8.87	<.01
B	34	28	15	9	18	.35	.75-.5
C	54	13	22	12	9	0.00	>.995
A (adjusted)	30	23	23	17	5	.11	.75-.5
Pooled A, B, C	118	51	73	38	32	.47	.5-.25
A (adjusted), B, C	118	64	60	38	32	.49	.5-.25

Nine  $F_3$  lines were examined (Table 2). Two of these lines derived from normal-green  $F_2$  plants, and seven from abnormal-chlorotic  $F_2$  plants. Evaluation of the  $F_3$  lines occupied a longer period of time than evaluation of the  $F_2$  populations, resulting in a lower degree of chlorosis in the  $F_3$ 's and greater difficulty in scoring. The two normal  $F_3$  lines (11 and 27) fit a 9:7 ratio for a two-gene model (Table 2), indicating they derived from double heterozygotes (i.e.,  $A B$ ). The chlorotic lines were expected to produce only chlorotic plants; however, no line fit solely into classes 3 through 5 (Table 2). Because of this unexpected result, and the difficulty encountered in giving chlorosis scores, five of the abnormal  $F_3$  lines were reevaluated (Table 3).

Table 2  
Distribution of chlorosis scores within  $F_3$  lines from  $F_2$  plants

F <sub>3</sub> line	Parental plant chlorosis score	Chlorosis score					χ <sup>2</sup> (9:7)	Prob- ability
		Normal		Abnormal				
		1	2	3	4	5		
Normal								
11	1		2	1	2	2	1.9	.25-.10
27	1	4		3	3		1.0	.50-.25
Abnormal								
1	5	2	2	2	3	1		
2	5		1	1	5	3		
3	5	2	2	3	2	1		
4	5	2	4	1	2	1		
5	5		3	2	2	2		
6	5	6	3			1		
7	5	7	2					

Table 3  
Reevaluation of chlorotic  $F_3$  lines 1, 2, 4, 6 and 7

$F_3$ line	Parental plant chlorosis score	Chlorosis score				
		Normal		Abnormal		
		1	2	3	4	5
Abnormal						
1	5			4	12	4
2	5			5	2	5
4	5			14	5	1
6	5	14		4		2
7	5		3	9		4

In the reevaluation, plants in lines 1, 2 and 4 were all classified in categories 3 through 5, but lines 6 and 7 still had plants that fell into categories 1 and 2. There are two possible explanations for the behavior of lines 6 and 7. First, the  $F_2$  parental plants identified as abnormal on the basis of chlorosis may have been misclassified. This may have also been the case with some of the  $F_3$  plants which fell into groups 1 and 2 in Table 2. The extra length of the first  $F_3$  experiment as compared with the other experiments may have led to this misclassification.

The second possibility is that the model fitted to the  $F_2$  data is incorrect. An attempt was made to fit a three-gene model to the data, but no good fit was found. Dinitrogen fixation is a complex trait and many different steps are involved before nitrogen is converted to a form usable by the plant. It is not unlikely that more than two genes could be causing the chlorosis observed in Amsoy 71.

In addition to chlorosis score, plant top dry weight, nodule weight, total nodule activity (TNA), and specific nodule activity (SNA) were measured on the  $F_2$  plants and on both Amsoy 71 and Anoka. Amsoy 71 showed a lower dry weight, nodule number, nodule weight and TNA than Anoka; however, SNA was not different for the two strain-variety combinations. Visual examination of the root systems of the  $F_2$  plants and of Amsoy 71 showed nodules to be normal in appearance with no discernible difference between normal and abnormal  $F_2$  plants. This, along with the fact that TNA levels of abnormal  $F_2$  plants and of Amsoy 71 were still appreciable, may indicate that chlorosis is not related to the rate of nitrogen fixation. The chlorosis may be associated with some other factor in the nodule.

The difficulties encountered in this study give some indication of the problems involved in studying nitrogen fixation. The abnormal nodulation observed and studied by other researchers has generally resulted in an almost complete lack of nitrogen fixation. Rates of nitrogen fixation for the Amsoy 71-strain 62 combination here were still significantly higher than zero. This obviously led to difficulty in scoring chlorosis in the plant since nitrogen fixation, as measured by chlorosis, may not be a good indication of what is actually occurring in the plant. Further work needs to be done before the chlorosis resulting from the Amsoy 71-strain 62 combination is fully understood.

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1) Mosaic resistant and susceptible soybean lines.

Isolines of soybeans are useful tools to study various interactions under field conditions. The purpose of this communication is to report the pending release of four pairs of mosaic resistant and susceptible soybean lines. These pairs of lines can be used in a variety of investigations dealing with soybean mosaic virus, and the resistant lines can serve as genetical material for plant breeders as sources of mosaic resistance.

Each resistant and susceptible sibling pair was selected as F<sub>3</sub> plants from the same F<sub>2</sub> plant from the second or third backcross to the recurrent mosaic-susceptible parent. Resistance, controlled by a single dominant gene, *R<sub>sv</sub>* (Kiihl, 1976), was obtained from soybean PI 96,983 from Maturity Group V of the soybean germplasm bank (Ross, 1969a).

The lines and their pedigrees are presented in Table 1. The lines have been used to study the effect of soybean mosaic virus on soybean yields (Ross, 1977). Results of field experiments with these lines have indicated among other things that (1) cv 'Dare', although infected by mosaic virus, possesses a field resistance to mosaic not present in 'Semmes', 'Pickett 71' or 'Lee 68'; (2) yields from Semmes may be reduced up to 39% by mosaic and yields of Lee 68 and Pickett 71 reduced 20-30%; (3) incorporation of mosaic resistance into soybean cultivars would be a worthy addition where mosaic is present. Average yield in the presence of soybean mosaic virus of the susceptible line from each pair was not significantly different ( $< + 4.5\%$ ) from yields of their respective recurrent parent in 1976 field tests at Plymouth, NC.

Table 1  
Pedigrees of mosaic resistant (R) and susceptible (S)  
isolines released

Line designation	Pedigree
NC-DMS } NC-DMR }	[(Dare x PI 96983) x Dare x Dare] x Dare
NC-SMS } NC-SMR }	[{(Semmes x PI 96983) x Semmes} x Semmes] x Semmes
NC-PMS } NC-PMR }	[[(Dare x PI 96983) x Dare} x Pickett 71] x Pickett] x Pickett 71
NC-LMS } NC-LMR }	[{(Lee 68 x PI 96983) x Lee 68} x Lee 68] x Lee 68



All pairs appear to have similar agronomic characters and disease reactions as those of the recurrent parent. Hence, NC-PMS and NC-PMR are resistant to Race 1 of Heterodera glycines, the soybean cyst nematode, as is Pickett 71. Since resistance to bean pod mottle virus has not been identified in the soybean germplasm, mosaic-resistant cultivars would not sustain the synergistic yield losses caused by double infection of pod mottle virus and mosaic virus (Ross, 1968, 1969b). Approximately 100 seed of each line may be obtained from J. P. Ross upon request.

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#### 1) Effects of light on soybean leaf chlorophyll content--The role of the $Y_{11}$ gene.

Previous studies on the genetics of chlorophyll production have revealed the involvement of a gene  $Y_{11}$ , which is incompletely dominant. Thus, three phenotypes may be observed--plants with leaves that are normally pigmented, light-green or yellow. The yellow is a lethal in nature; however, we have propagated them under laboratory conditions either by grafting the yellows to wild-type plants or growing them independently under constant low-level illumination with a short period of moderate (400 ft-c) illumination each day. Under the low light conditions, the presence of considerable chlorophyll is evident in the leaves of these yellow plants (Noble et al., 1977). Such plants are capable of sufficient  $CO_2$  fixation to survive and grow at a reduced rate.

Variations of the light environment have revealed that the chlorophyll content of the light-green phenotype can be increased by 100% but this same lighting condition increases the chlorophyll content of dark-greens by less than 20%. Furthermore, chlorophyll content of yellows can be elevated 900%.

From the data in Table 1, it is seen that yellow plants can be grown with chlorophyll levels as high as those for light-greens grown under normal conditions. Visual distinction between the two cannot be made on the basis of leaf color, but can be made on the basis of plant vigor. Visual distinction between dark-green plants grown under high light and light-green plants grown under low light is not usually possible.

We first noted the effects of light intensity on chlorophyll content of leaves in 1972 when our first grafting studies were done; however, such effects were not reported in the literature until later. Koller and Dilley (1974) reported increases in chlorophyll content in the light-green with decreasing light intensity. They did not approach a condition where the light-green plant had chlorophyll levels as high as those in dark-green plants.

These observations point to the likelihood that the  $Y_{11}$  gene is not directly involved in the biochemical pathway leading to chlorophyll synthesis. Instead it appears to be involved either in the regulation of the amount of chlorophyll synthesized or the regulation of the rate of degradation of chlorophyll following synthesis.

Table 1  
Chlorophyll content (mg per gram fresh weight) of three  
phenotypes vs. light intensity

	Dark-green		Light-green		Lethal yellow	
	High light intensity	Low light intensity	High light intensity	Low light intensity	High light intensity*	Low light intensity
Chlorophyll	2.04	2.38	.6	1.45	.09	.62
Number plants	9	5	5	5	7	7

\*Grafted.

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- Koller, H. R. and R. A. Dilley. 1974. Light intensity during leaf growth affects chlorophyll concentration and  $CO_2$  assimilation of a soybean chlorophyll mutant. *Crop Sci.* 14: 779-782.
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## 2) Photosynthetic activity in chlorophyll deficient soybean leaves carrying the $Y_{11}$ mutant.

In soybeans, the  $Y_{11}$  gene is involved in chlorophyll synthesis. Thus, in the heterozygous condition  $Y_{11}y_{11}$ , an intermediate or light-green leaf pigmentation results. Photosynthetic  $CO_2$  assimilation in the light-green, on a surface area basis, has been reported to be as high or nearly as high as in the homozygous dominant, dark-green plant (Wolf, 1965; Keck *et al.*, 1974; Cappy and Noble, 1974; Crang and Noble, 1978). When photosynthesis is expressed on a chlorophyll basis, the rates for light-green plants are quite impressive. Koller and Dilley (1974) report photosynthesis to be four times greater in light-green than in dark-green plants, when expressed on a chlorophyll basis.

Such observations led Stiehl and Witt (1969) and Keck *et al.* (1970) to the hypothesis that the light-green phenotype might possess a more efficient energy trapping system. They went to the rate limiting step in the electron transport system and were able to show a substantially faster rate of oxidation of plastiquinone in pigment systems from light-green leaves. These observations seemed to confirm the notion that the efficiency of the photosynthetic system of the light-green was greater than that of the dark-green phenotype.

Our own observations reveal that photosynthesis in the light-green plant is three to four times faster than in the dark-green (on a chlorophyll basis); however, when expressed on a surface area basis, the rate of  $CO_2$  uptake in the light-green was 15-20% lower. These measurements were made on plants grown at 2500 ft-c. In an attempt to test the photosynthetic efficiency of the light-green plants further, they were grown under continuous incandescent illumination, at 60 ft-c, with a supplemental four-hour period of fluorescent illumination at 400 ft-c.

Under these conditions, the chlorophyll content of the light-green plants rose from 0.6 mg (on a gram fresh weight basis) to 1.45 mg, and an inverse photosynthetic relationship was observed. When light-green plants from low light and high light conditions were compared on a chlorophyll basis, the photosynthetic rate dropped from 15.4 mg  $CO_2$  to 9.2 mg  $CO_2$  while, on a surface area basis, the photosynthetic rate remained unchanged. If one uses the photosynthetic rates based on chlorophyll for the light-green phenotype to predict the photosynthetic rate for a dark-green whose chlorophyll content is known, the predicted and measured values coincide very closely. This suggests that the chlorophyll is functioning in a similar manner in both phenotypes.

From these observations, it appears that the amount of chlorophyll in the light-green phenotype is usually sufficiently high that it does not limit photosynthesis. Elevation of chlorophyll content, while not affecting net  $CO_2$  assimilation, results in lower efficiency when calculated on a per chlorophyll basis. Therefore, photosynthetic comparisons often made between the light-green and dark-green phenotypes may be misleading when made on this basis.

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1) A breeding project aimed at producing major morphological changes required to fit a soybean "idiotype".

There is evidence that only small increases in yield have resulted from soybean breeding in the United States during the past 30 years and that some major limitations to yield have been reached (Frey, 1971). During this period the main advances have been in developing resistance to pests and diseases, and improving agronomic traits such as resistance to lodging and shattering.

Considerable increases in yield have been achieved during this period in programs conducted for the development of soybeans in the tropics and sub-tropics through adaptability to short days and high temperatures. These yield improvements are analogous to those obtained in the U.S.A. some decades ago when comparable advances were made. It can therefore be expected that yield improvement will decline once certain levels have been reached.

What factors limit yield? The physiological limitations to yield which have been reached probably relate to both the carbon and nitrogen metabolism of the crop. It is necessary to examine some information relevant to these limitations before considering how they may be overcome.

Carbon assimilation as a limitation of yield: The photosynthetic apparatus of this crop is not remarkable for its efficiency, and in the opinion of many researchers in this field, there is little prospect of large yield improvements (Duncan, personal communication). The plant has a C<sub>3</sub> metabolic pathway and therefore individual leaves become light saturated at relatively low light intensities. While there are variations in photosynthetic rate between varieties (Dornhoff and Shibles, 1970; and Shibles, personal communication), the relationship between yield and photosynthetic capabilities is apparently not great.

It would seem, therefore, that the problem of photosynthate supply may best be overcome by spreading the available light at a lesser flux density



over a larger area of leaf. This would require modification of the existing canopy structure. The establishment of a powerful reproductive sink may also increase photosynthesis since increased demand has been shown to increase supply of assimilates. This phenomenon may exist for soybeans (Dornhoff and Shibles, 1970), although the extent and limit of this stimulation has not been defined. It may be a sizeable increase since a two week improvement of light into the lower regions of the canopy at the pod setting stage may increase yield by as much as 40% (Schou *et al.*, 1978). The nutrient limitation to yield would therefore appear to be the one which is of greatest significance to yield.

Nitrogen as a limiting factor to yield: The role of nitrogen as a limiting factor in the determination of yield potential has been researched by a number of workers since Sinclair and de Wit (1975, 1976) concluded that the seeds accumulated nitrogen at a rate in excess of the crop's ability to achieve N accumulation and utilized N from the leaf to achieve this. This use of nitrogen from the leaves reduces photosynthesis and is associated with rapid senescence (Murata, 1969; Egli *et al.*, 1978).

Attempts to overcome this by foliar applications of nutrients have been consistently successful only in greenhouse experiments (Hanway, personal communication).

This problem may also be overcome by increasing the supply of carbohydrates to the roots and nodules. Nodule activity is closely dependent on the supply of assimilates to the roots (Hardy and Havelka, 1976). Any change in photosynthate availability has a rapid effect on the nitrogen fixation by the nodules which is greatly reduced once the pods start to grow. The roots appear to be unable to compete with the pods for the carbon assimilates for the following reasons. First, the distribution of assimilation within the plant is usually from the leaves to the nearest sinks and the pods are nearer to the leaves than are the roots. Second, the lower leaves which are normally responsible for the carbon nutrition of the roots have either senesced or are in very poor radiation conditions and unable to support the roots with the amounts of carbohydrates that they need for active N fixation. The consequence of decreased root activity to the plant may extend beyond the reduced mineral assimilation since the roots also produce cytokinins which are involved in the senescence of the leaves (Torrey, 1976).

Plant morphology and competition: Individually soybean plants have a capacity to yield substantially more than they do in a crop community. The competition afforded by neighboring plants reduces the nitrogen fixation by the plants (Weil and Ohlrogge, 1975) and this becomes the yield limiting factor and results in hastened senescence (Egli *et al.*, 1978). When the soybean plant is examined in the light of the above, the petioles and their development are a major disadvantage to the plant. The petioles of normally spaced plants are relatively short at the bottom of the plant and increase in size progressively up the plant until those near the top reach a maximum size in excess of 30 cm. These petioles spread the leaf away from the central axis of the plant and effectively shade the lower leaves. This has the undesirable consequence for the supply of carbohydrates to the nodules that has been described earlier.

The petioles also constitute about 33% of the growth prior to pod filling and potentially this material could be utilized elsewhere by the plant and could contribute to increased seed yield.



Is it possible to overcome these limitations? This question remains unanswered at present although it has been hypothesized that changed morphology may help to overcome these problems by improving light into the lower canopy. This will require changed petiole characteristics.

Plant breeding for improved morphology: No genotypes with sessile or near sessile leaves have been found among our collection. The possibility that such a type may arise through mutation led to an irradiation project. Air-dry seed of cv. 'Rhosa' were exposed to three levels of gamma radiation (6,000, 12,000 and 18,000 r) using a Cobalt 60 source. The irradiated seed was then planted in the field, and regularly inspected to find any mutant of interest to the program.

Among the plants from the 18,000 r treatment one was found with the petioles from the first nodes being normal in length and becoming progressively shorter up the stem until the top leaves are almost sessile, creating a 'pine tree' shaped canopy. This plant produced 15 seeds which have been grown as separate lines for three generations. No segregation occurred for the main abnormalities of the original selection which were, in addition to the smaller petioles, crinkled leaves and decreased plant height.

The mutant was undesirable from two aspects in that it was dwarfed and produced fewer seeds than normal plants. In order to improve these defects and also to establish the mode of inheritance of the mutation, three crosses were made to well-adapted prolific lines. In two of these crosses the mutant was used as the female parent and in the other it was the male parent. In all crosses the  $F_1$  plants were normal and in the  $F_2$  segregation was as shown in Table 1.

Table 1  
Segregation ratios in the  $F_2$  generation of progeny of crosses  
between mutant and normal parents

♀ parent	♂ parent	Normal	Sessile	Chi-square	Probability of ratio being 3:1
74/6/23	Mutant	165	13	29.73	p = 0.01
118/6/40	Mutant	41	37	13.64	p = 0.01
Mutant	20/6/25	280	89	0.14	p = 0.7-0.8

In one cross the mutant behaved as a simple recessive but not so in the other two crosses. At this stage it seems that the mutation is recessive and not cytoplasmic but just how many loci are involved is not clear and further investigation is indicated.

Of practical interest, however, is that the types of plants selected from these crosses appear to have considerable promise. Single plant selections were taken at the  $F_2$  and further selections of their progeny in the  $F_3$ . These plants, selected for normal height and a 'pine tree' canopy at the early

reproductive stage, have proved to be high yielding. This tends to confirm the importance of canopy modifications to future improvements of soybean yield. The improved light penetration associated with the canopy change has resulted in heavy podding in the middle and lower strata of the crop. Leaf area duration has been increased and the plant structure seems less likely to lodge. The height of the lower nodes appears to have been unaltered.

Further selection work must continue to stabilize these lines and the benefits of the mutant form must still be demonstrated in yield trials. However, it does appear that a single cross with the mutant onto a suitable genotype can produce desirable plant types which should have certain desirable physiological properties not normally found in the species. On the arguments presented in this note, and our own initial observations, this may well lead to increased seed yield.

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1) Cross pollination studies of soybeans using a genetic male sterile system.

Information regarding cross pollinating insects in soybeans has been mainly restricted to honeybees. Erickson (1975) reported that attractiveness of soybeans to honey bees appeared to be heritable. Jaycox (1970) reported on the ecological relationships between honey bees and soybeans.

In 1977 we started a cross pollination study using alfalfa leaf cutter bees (Megachile rotundata) as the pollination insect on an F<sub>3</sub> population of genetic male sterile soybean plants.

The genetic male sterile plants were derived from a complex cross,

$$\frac{('Viking' \times 'Classic II') F_1}{('Mitchell' \times 'Columbus') F_1}$$

This genetic system segregated as a simple recessive in this population. The gene for genetic sterility was traced to the variety 'Columbus'.

From the greenhouse in 1976 a series of white flowered, grey pubescence plants from the F<sub>2</sub> generation was selected as female parents for this study.

Seed from these selected plants were blended with a purple flowered, brown pubescent male parent 'RA-427' in a 1:1 ratio. A total of nine rows 20' long in 40" rows were planted adjacent to the soybean nursery in Plainview in 1977. Seedling emergence in the F<sub>3</sub> grey pubescence, white flowered plants and the brown pubescent, purple flowered male RA-427 (early 5 maturity line) was excellent.

At flowering time in late June Mr. Van der Vliet rogued all fertile white flowered, grey pubescent plants from the nine rows using a microscope as final determination. The expected 3:1 ratio was not achieved due to our inability to recognize the heterozygous F<sub>2</sub> plants in the greenhouse and some homozygous fertile plants were included. A total of 35 plants having the desired sterility, grey pubescence and white flowers were saved and allowed to cross with any available male in the nursery as well as the adjacent RA-427 plants. A total of 430 grams of F<sub>1</sub> seed was obtained. Many of the genetic sterile plants set nearly normal amounts of seed and the usual late maturity noticed in many sterile plants was not present. Summer observations were made for flower visitation by alfalfa leaf cutter bees. A large number of these bees were noticed visiting soybean flowers in the nursery and on the nine rows having male sterile plants. Some ground dwelling bees, mainly Agropostemon texanus and Halictus ligatus also were observed near and on the soybean flowers. No honey bees were found in the soybean nursery.

A total of 400' of these F<sub>1</sub> plants were grown out in 1978. The hoped-for crossing between the grey pubescent, white flowered sterile plants and the brown pubescent, purple flowered RA-427 adjacent plants occurred only 50% of the time. The resulting F<sub>1</sub> hybrids were an unexpected mixture of F<sub>1</sub> plants having many characteristics such as tall F<sub>1</sub>'s grey pubescence, brown pubescence, etc., indicating a wide diversity of male donors to the 1977 crossing block.

Observations in 1977 and 1978 on soybean plants in Plainview, Texas, indicated that leaf cutter bees were active on soybean leaves to obtain the necessary round plugs for their egg laying activities in the domiciles provided.

From these experiences one could conclude that it would be feasible to use the alfalfa leaf cutter bee, *M. rotundata* to effectively cross-pollinate sterile soybean plants, but that these plants would have to have considerable isolation from other soybeans except for the chosen male parent.

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### 1) The influence of low temperatures on the development and structure of yield formation of three cold tolerant and a standard soybean variety.

The amount and stability of the yield of soybeans cultivated under Swiss climatic conditions is still unsatisfactory. Breeding studies (Piattini, 1977; Soldati, 1976) in relation to yield structure under various climatic conditions in Switzerland were conducted. It became evident that poor utilization of the available yield potential of different soybean varieties could be attributed mainly to low temperatures in the course of the vegetation period. Therefore, under Swiss temperature conditions, we investigated the cold tolerance of three cold tolerant varieties, 'Amurskaja 41' (Russia), 'ISZ-7' and 'I-1' (Hungary), and a standard variety 'Gieso' (Germany), well-adapted to our climate. In this way, the basic work for further breeding, taking cold tolerance into consideration, should be established.

Cold tolerance cannot only be considered with reference to the early stages of development; it is also of great importance during other stages, especially flowering. Cold tolerance behavior was investigated in growth chamber, greenhouse and field experiments with reference to the following three factors: (1) influence of the moment of the cold stress in the course of vegetative and reproductive development; (2) influence of the duration of the cold stress; and (3) influence of the temperature levels.



Sensitivity to temperature of two soybean varieties (Amurskaja 41 and Gieso) in the course of vegetative and reproductive development: As one of the treatments under glasshouse conditions, the temperature was shifted every 10 days from the beginning of sowing, from the high level (25/17°C) to the lower level (20/14°C day/night temperature) for 4 or 14 days.

Vegetative growth was retarded immediately due to the decrease in temperature. This led to a compensation reaction in which, for example, the plants under stress formed longer internodes in the upper part of the main stem.

The yielding reaction is, therefore, conclusive for an assessment of the cold sensitive stages of the soybean. Even 4 days of cold stress during various time periods of vegetative and reproductive development led to a great variation in yield for both varieties. The rather cold sensitive Gieso showed an increase in cold sensitivity from vegetative stage VI (Fehr and Caviness, 1977) through V3 until the start of flowering. Amurskaja 41 produced a significantly higher yield with the plants which endured cold stress during these stages than did Gieso. Astonishingly, the highest yield (20 g/plant) was produced by Gieso and Amurskaja 41 when the stress occurred at the beginning of pod formation. Plants under constantly high temperatures (25/17°C) did not achieve this yielding level, probably due to the need for changing temperatures. Constantly cool conditions reduced the yield of Gieso in contrast to a stable warm environment. Amurskaja 41 reacted differently in that it produced higher yields under cooler conditions. The various yielding reactions can be explained by the differences in pod and grain number as well as the hundred seed weight.

The effect of the duration of the cold stress: Within a glasshouse environment plants of the Gieso, Amurskaja 41, ISZ-7 and I-1 varieties were subjected to cold stress during the V1, V3 and R1 stages of development. This lasted for a period of 10 days or until maturity. The upper and lower temperature levels were readjusted monthly: high temperature variant--18/12, 21/14, 23/16, 23/18 and 22/15°C; low temperature variant--12/7, 16/12, 19/13, 22/16 and 20/15°C day/night temperature.

Vegetative development was greatly retarded or even stopped by the cold stress. The compensation reaction to a short period of stress followed relatively quickly. A longer period of stress could be compensated for only later, and only the cold tolerant varieties were able to compensate fully. Dry matter production per plant (excluding roots) showed that ISZ-7 produced as much dry matter under a lasting cold stress as did Gieso in the warm control. The pod set, expressed as percentage and based on the maximum number of flowers, was significantly higher for plants of the I-1 variety which had been subjected to a long stress period in all three sensitive stages of development (V1, V3, R1) as compared with the Gieso variety. The compensation ability was exceeded for the Gieso and Amurskaja 41 varieties at these temperatures. The high stability of ISZ-7 and I-1 under extreme temperatures was expressed by the Harvest Index. ISZ-7 and I-1 demonstrated their cold tolerance characteristics by producing significantly higher yields under a long period of cold stress as opposed to Gieso and Amurskaja 41. The compensation ability of these varieties was clearly calculated on the basis of the coefficients of variation with regard to the yields of all cold stress treatments including the controls in the high and low temperature variants: Gieso, 55.4%; Amurskaja 41, 79.1%; ISZ-7, 29.8%; I-1, 29.6%.



Influence of temperature levels: After a 15-day cultivation period at 20/15°C day/night temperatures, the Gieso, Amurskaja 41, ISZ-7 and I-1 varieties were planted under the following three temperature regimes, within the scope of a growth chamber experiment:

Temp. regime	14 days	4 days	26 days	23 days	7 days	31 days	30 days	
1	14/8	18/12	22/16	24/17	23/16	21/16	18/13	= warm (°C)
2	11/6	15/10	19/13	21/15	23/16	21/16	18/13	= cool (°C)
3	6/2	11/6	15/10	19/13	19/13	21/16	18/13	= cold (°C)

The vigor of the cold tolerant varieties ISZ-7 and I-1, under the coldest temperature regime, was especially evident in the increased average growth rate of the main tiller 70-100 days after sowing, as opposed to Gieso and Amurskaja 41, ISZ-7 and I-1 did not exhibit a higher cold tolerance during the early stages, but were, however, better able to make good use of the subsequent higher temperatures than were Gieso and Amurskaja. The cold influence provoked a considerable delay of flowering and maturity. The relatively high correlations between the daily temperature sums in the developmental stages from the beginning of flowering up to the beginning of grain formation and the yield, verifies the great importance of this developmental period under cold temperatures. The I-1 variety exhibited a relatively high rate of pod formation within the cold variants (2 and 3) owing to a great increase in pods up to maturity, or because of a marked rise in the number of flowers which brought about the addition of a relatively high number of pods even after flower drop. The I-1 and ISZ-7 varieties are much more capable of utilizing the cold temperatures for grain/dry matter production than are Gieso and Amurskaja 41 (Table 1). Whereas all varieties produced a similar yield under warm temperatures, ISZ-7 showed a moderate and I-1 a pronounced (significant) tendency to increase their grain yields under cool and cold temperatures. These increased yields can be attributed to the high number of pods and grains as well as to the hundred seed weight. In contrast, Gieso and Amurskaja 41 exhibited a strong decrease in yield under cooler temperature conditions.

Table 1

Grain weight (dry matter) per °C (mg/°C) with reference to the daily temperature sums from sowing to maturity (LSD 5% = 1.024)

Temp. regime	mg/°C			
	Gieso	Amurskaja 41	ISZ-7	I-1
warm	5.7	5.2	5.4	5.4
cool	4.7	5.0	5.3	6.2
cold	2.1	3.1	5.5	6.7

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### 1) Studies on Phytophthora rot in soybeans.

The cultivar 'Tracy' had been extensively used as a parent in breeding programs even before Laviolette and Athow (1977) reported that it was resistant to all nine of the identified races of Phytophthora megasperma Drech. var. sojae Hildeb. Kilen (1977) reported that Tracy had two major genes for resistance to race 1 of the pathogen. His data, based upon segregation of the F<sub>2</sub> generation, also suggested that the two genes occupied loci different from Rps<sub>1</sub> and Rps<sub>2</sub>. This paper is a report on the reaction of (1) F<sub>2</sub> populations from the crosses 'Pickett 71' x Tracy and Tracy x D60-9647 inoculated with race 1; and (2) F<sub>3</sub> lines from the crosses Tracy x Pickett 71 and Pickett 71 x Tracy inoculated with races 1 and 2 of the pathogen. Pickett 71 and D60-9647 have different alleles for resistance at the Rps<sub>1</sub> locus. The reaction of D60-9647 is the same as that for 'Sanga', and they probably have the same resistance alleles.

Each F<sub>3</sub> line was evaluated by the reaction of 10 to 12 plants. Because this was too small a sample to distinguish uniformly resistant from segregating families, emphasis was placed upon the uniformly susceptible families. The hypocotyl puncture method of inoculation was used. After inoculation, the seedlings were kept in a moist chamber overnight. Notes on disease reaction were taken about 5 days later.

There was no segregation in the F<sub>2</sub> generation of either cross when inoculated with race 1 (Table 1). These results suggest that one of the genes for resistance in Tracy is at the Rps<sub>1</sub> locus. Similarly, there were no susceptible or segregating F<sub>3</sub> lines when inoculated with race 1 (Table 2).

Table 1

Reaction of two  $F_2$  populations, parents and a susceptible strain inoculated with race 1 of the pathogen

Cross or strain	Number of plants	
	Dead	Alive
(Pickett 71 x Tracy) $F_2$	0	372
(Tracy x D60-9647) $F_2$	0	388
D55-1492	20	0
Pickett 71	0	19
Tracy	0	20
D60-9647	0	18

Table 2

Reaction of  $F_3$  lines, their parents and two differential strains, inoculated with races 1 and 2 of the pathogen

Cross or strain	Number of lines or plants					
	Race 1			Race 2		
	R <sup>+</sup>	SEG	S	R	SEG	S
Tracy x Pickett 71	100	0	0	62	31	7
Pickett 71 x Tracy	100	0	0	69	26	5
D55-1492	0		20	0		29
Pickett 71	19		0	20		0
Tracy	20		0	20		0
D60-9647	18		0	1		42

<sup>†</sup>R = resistant; SEG = segregating; S = susceptible.

The reaction of the  $F_3$  lines when inoculated with race 2 indicates that one of the genes in Tracy gives a similar reaction to races 1 and 2 as the gene for resistance in D60-9647 (Table 2). Both crosses segregated at about a 15:1 ratio if the resistant and segregating lines are pooled. These results are consistent with those expected if one gene in Tracy is resistant to both races 1 and 2, and the second gene is resistant to race 1 but susceptible to race 2.

Both D60-9647 and PI 171,442 are in the ancestry of Tracy. Race 2 was used to screen the  $F_3$  lines from which Tracy was selected. At that time, it was assumed that the alleles for resistance in PI 171,442 and D60-9647 were at the same locus. It was therefore assumed that the selection of lines uniformly resistant to race 2 would eliminate the alleles from D60-9647. We now

know that the gene for resistance from PI 171,442 could mask the effect of the gene from D60-9647. It therefore seems likely that Tracy has one gene from PI 171,442 and one gene from D60-9647, or that Tracy has two genes from PI 171,442, one of which acts like the gene in D60-9647. However, we have not been able to detect a second gene for resistance in PI 171,442. The appropriate crosses to properly identify the genes for resistance in Tracy have been made at this and at least one other research station. Such information will enable soybean breeders using Tracy as a parent to select the combination of races that will improve the efficiency of screening for resistance to phytophthora rot.

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1) Pollen movement to male sterile soybeans in southern Illinois.

The effective use of genetic male sterility in soybeans requires controlled pollen movement. Soybean pollen is carried by insects with bees usually considered the most likely candidate in Illinois. The following experiment was designed to determine how far soybean pollen can be carried to fertilize male sterile soybeans when pollen is also available from adjacent plants.

Equal amounts of 'Williams', a white flowered variety, and a backcrossed line of Williams containing  $ms_2$  were mixed together and planted in 75 cm rows in a plot 30 m long and 15 m wide. The male sterile line was segregating in a 6:1 ratio for male fertility and male sterility. The plot was bordered on the south and east by grass which was mowed throughout the summer and no crops were planted for at least one hundred meters in either of those directions. On the other two sides 'Calland', a purple flowered variety which matures similarly to Williams, was planted. The experiment was conducted at the S.I.U. Belleville Research Center east of St. Louis, Missouri.

At harvest each of the 20 rows was divided into ten 3 m segments and seeds from all male sterile plants within each segment were bulked. The seeds were germinated in the greenhouse, and the percent of purple hypocotyl seedlings was determined. For the purpose of calculation, all distances are measured from the edge of the Calland planting to the center of the area under consideration. In total, 268 male sterile plants were harvested with an average of 27 seeds per plant.

Table 1 lists the percentage of Calland-pollinated seeds from 50 areas of the field. Each area is 3 m long and 4 rows or 3 m wide. This table is arranged so that by moving from left to right, the distance across-rows from Calland increases and by moving from top to bottom the distance within the row from Calland increases. The means for within-row distances are given in the far right column and the means for across-rows distances are given on the bottom line. These data are graphically represented in Figure 1 by simple linear regression of the percent Calland-pollinated seeds on the distance from the Calland pollen source measured for both within- and across-rows distances.

The decrease in Calland-pollinated seeds as the distance across-rows increases was expected. The regression equation of  $Y = 7.53 - 0.523X$  (Fig. 1) is very similar to one reported by Boerma and Moradshahi (1975) in Georgia ( $Y = 6.46 - 0.476X$ ). The within-row data seems to be inconsistent, since the percent of Calland-pollinated seeds reaches a minimum at 13.5 m and then steadily increases until at 28.5 m it is approximately 40% of the 1.5 m value. The simple linear regression line has very little slope ( $-0.106$ ) and examination of the data points indicates a quadratic response (Fig. 1). However, if only the data for the first 15 m are included, the regression equation is  $Y = 7.41 - 0.497X$  which is almost identical to the across-rows regression equation (Fig. 1).



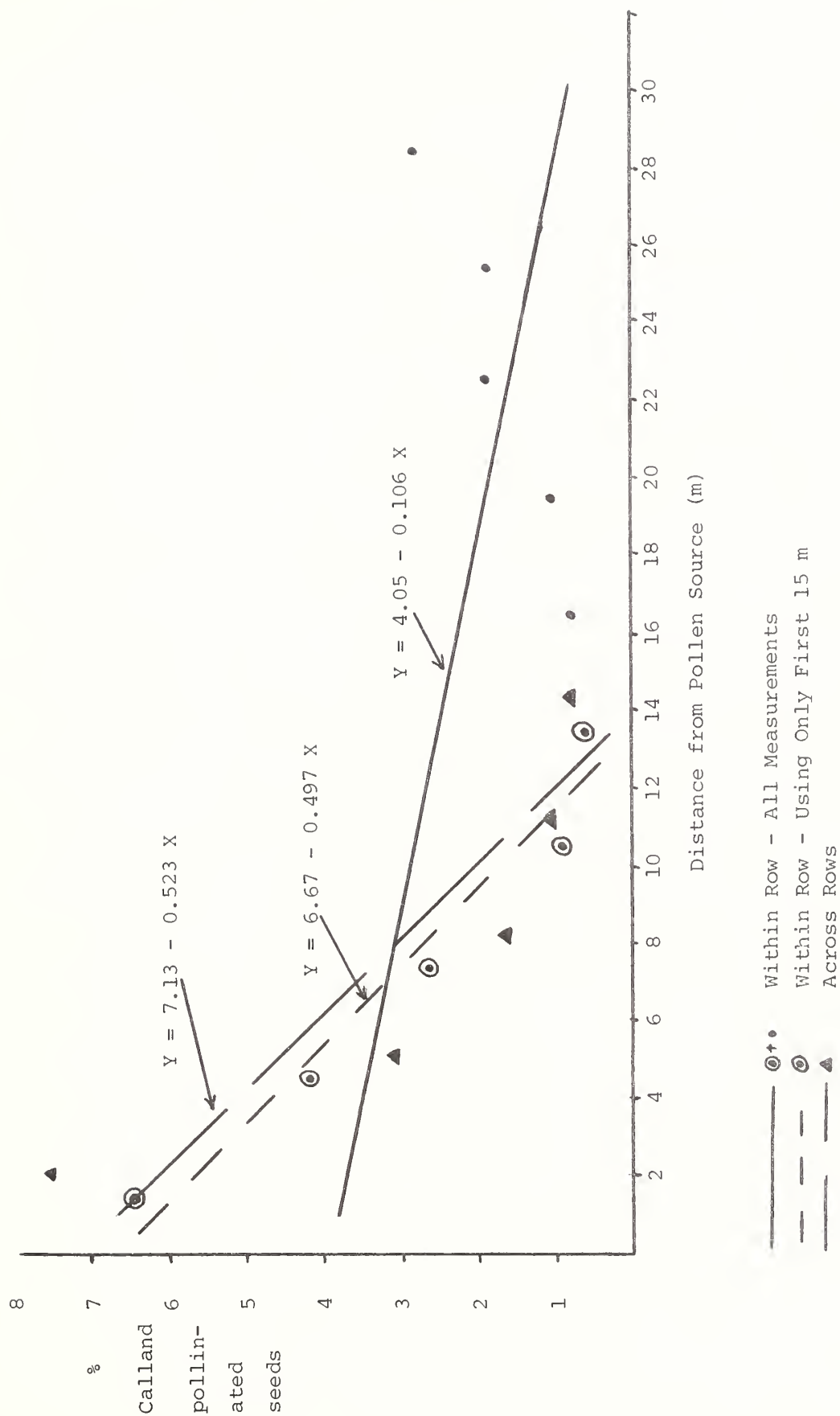


Fig. 1 Mean Percentage of Calland Pollinated Seeds from Williams-ms2 ms2 Plants for Within and Across-Rows Distances from Calland Soybeans

Table 1

Percent of Calland pollinated seeds from seeds  
harvested from Williams  $ms_2ms_2$  plants

Distance from Calland within-row	Distance from Calland across rows (m)					Mean % of Calland pollinated seeds	
	2.25	5.25	8.25	11.25	14.25	All rows	Rows within 5m of Calland omitted
C A L L A N D							
1.5	15.9	12.1	6.8	1.7	4.0	6.4	5.4
4.5	8.9	3.9	7.5	0.0	0.0	4.2	2.9
7.5	C 10.2	3.4	1.6	0.0	1.2	2.6	1.7
10.5	A 7.1	2.1	0.0	0.0	0.0	G 0.9	0.3
13.5	L 3.1	0.0	0.0	0.0	0.0	R 0.6	0.0
16.5	L 4.0	3.2	0.0	0.0	0.0	A 0.8	0.4
19.5	A 17.2	0.0	1.5	0.0	0.0	S 1.0	0.2
22.5	N 3.4	2.9	0.0	1.3	1.3	S 1.8	1.4
25.5	D 5.6	0.0	0.5	2.3	0.0	1.8	0.9
28.5	9.2	0.0	0.8	0.5	1.0	2.8	0.7
G R A S S							
Mean % of Calland- pollinated seeds	7.6	3.1	1.6	1.0	0.8		

The grass borders were chosen to be a neutral area which would allow soybean pollen to enter the male sterile population from only two directions. However, it seems as if the grass had a positive influence on pollen movement. A cursory examination would suggest that the effect is seen only in the within-row measurements, but if the mean percent of Calland-pollinated seeds is calculated for within-row distances omitting the first column of Table 1, or the across-rows distance closest to Calland, the linear increase between 15 and 30 m disappears (Table 1). This suggests that the pollen vectors are more active near the grass, but that the percentage of pollen contamination increases noticeably in those areas which are close to both the grass and the pollen source. This is evident in both corners of the Williams block where Calland adjoins the grass. There is some evidence that pollen moves more freely within the row than across-rows which may help explain the more pronounced effect on the within-row measurements (Boerma and Moradshahi, 1975; Jaycox, 1970). Also the pollen vectors may have been moving from the pollen source (Calland) into the grass and then back into the male sterile block which could account for the small areas of relatively high pollen contamination in the corner of the plot farthest from the pollen source.

It seems that for a plot completely surrounded by soybeans the data for the first 15 m in both directions would be applicable, with the exception that the area which is adjacent to the grass may be inflated. These data suggest that a buffer area of approximately 10 m of soybeans should eliminate almost all pollen contamination into a male sterile intermating block in southern Illinois, and a 5 m buffer area would allow less than 5% contamination.

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2) Five marker genes independent of  $ms_2$ .

In 1976 several crosses were made to determine if any linkage existed between  $ms_2$  and selected genes from the Genetic Type Collection (Bernard and Weiss, 1973).

The results from the  $F_2$  generation are presented in Table 1 with  $a = XY$ ,  $b = xY$ ,  $c = Xy$  and  $d = xy$  for the gene pairs listed in the form  $Xx$  and  $Yy$ . The ratio of products method (Immer and Henderson, 1943) was used to determine the percentage recombination.

$F_2$  ratios were also counted for the cross T259 x T256 ( $df_4$ ); however, no double recessive class was observed so no ratio of products could be calculated. By the use of  $\chi^2$  it was determined that an unusual segregation for  $Ms_2$   $ms_2$  had occurred but no linkage was detected. The crosses involving  $y_{10}$  and  $y_{13}$  also had fewer than expected male sterile plants which affected the ratio of products, but the  $\chi^2$  test gave no indication of linkage.

Table 1  
 $F_2$  linkage tests

Genes	a	b	c	d	Sum	%R $\pm$ SE	Linkage phase
T259 ( <u>Ln</u> <u>Ln</u> <u>ms<sub>2</sub></u> <u>ms<sub>2</sub></u> ) x T41 ( <u>ln</u> <u>ln</u> <u>Ms<sub>2</sub></u> <u>Ms<sub>2</sub></u> )							
<u>Ln</u> <u>ln</u> <u>Ms<sub>2</sub></u> <u>ms<sub>2</sub></u>	102	33	33	18	163	52 $\pm$ 5.7	R
T259 ( <u>Y<sub>10</sub></u> <u>Y<sub>10</sub></u> <u>ms<sub>2</sub></u> <u>ms<sub>2</sub></u> ) x T161 ( <u>y<sub>10</sub></u> <u>y<sub>10</sub></u> <u>Ms<sub>2</sub></u> <u>Ms<sub>2</sub></u> )							
<u>Y<sub>10</sub></u> <u>y<sub>10</sub></u> <u>Ms<sub>2</sub></u> <u>ms<sub>2</sub></u>	135	21	5	2	163	> 55	R
T259 ( <u>Y<sub>13</sub></u> <u>Y<sub>13</sub></u> <u>ms<sub>2</sub></u> <u>ms<sub>2</sub></u> ) x T230 ( <u>y<sub>13</sub></u> <u>y<sub>13</sub></u> <u>Ms<sub>2</sub></u> <u>Ms<sub>2</sub></u> )							
<u>Y<sub>13</sub></u> <u>y<sub>13</sub></u> <u>Ms<sub>2</sub></u> <u>ms<sub>2</sub></u>	184	23	18	1	226	39 $\pm$ 5.5	R
T259 ( <u>Wm</u> <u>Wm</u> <u>ms<sub>2</sub></u> <u>ms<sub>2</sub></u> ) x T235 ( <u>wm</u> <u>wm</u> <u>Ms<sub>2</sub></u> <u>Ms<sub>2</sub></u> )							
<u>Wm</u> <u>wm</u> <u>Ms<sub>2</sub></u> <u>ms<sub>2</sub></u>	83	18	22	4	127	48 $\pm$ 6.8	R
T259 ( <u>Lf<sub>2</sub></u> <u>Lf<sub>2</sub></u> <u>ms<sub>2</sub></u> <u>ms<sub>2</sub></u> ) x T255 ( <u>lf<sub>2</sub></u> <u>lf<sub>2</sub></u> <u>Ms<sub>2</sub></u> <u>Ms<sub>2</sub></u> )							
<u>Lf<sub>2</sub></u> <u>lf<sub>2</sub></u> <u>Ms<sub>2</sub></u> <u>ms<sub>2</sub></u>	72	30	12	7	121	55 $\pm$ 6.4	R

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### 1) Selection of a maternally inherited male-sterile trait in soybeans.

The induction of male sterility in soybeans with the use of ethidium bromide (EB) was reported in 1977 (Burton, 1977). Further investigations have provided evidence that the sterility of one of the plants recovered from mutagenesis is maternally inherited.

In 1976, large samples of 'Ransom', 'Jackson' and 'Lee 74' seeds were treated with EB and planted in the field (the  $M_1$  generation). Twelve phenotypically male-sterile plants, 7 Ransom, 4 Jackson and 1 Lee 74, were selected from this population (Burton, 1977). The  $M_2$  progeny from these plants were presumably hybrids, having a random fertile genotype as the male parent. These progeny were expected to be sterile if the induced  $M_1$  sterility was due to cytoplasmic factors, provided a dominant fertility restorer gene had not been contributed by the male parent. The progeny were expected to be male-fertile or a mixture of sterile and fertile if the induced  $M_1$  sterility was due to a single dominant nuclear gene or due to environmental factors.

Seed from the 12 plants selected in 1976 were planted in the field in 1977 (the  $M_2$  generation). Eleven of the 12 had fertile progeny. The other, a selection from Ransom, had only five progeny which survived to maturity, and all had phenotypes characteristic of genetic male-sterile ( $ms_1ms_1$ ) plants (reduced pod set, mostly one-seeded pods, and they remained green past the normal Ransom maturity). In addition, all of the plants had leaves with more than three leaflets. The seed from these plants were presumably hybrids with an unknown male parent. The plants were bordered on either side by male-fertile Ransom plants which should have increased the likelihood that Ransom was the male parent.

Three or four seeds from each plant were grown in the greenhouse during the 1977-78 winter (the  $M_3$  generation). The multi-leaflet trait was not expressed in these plants. At maturity, 15 plants had pods and 3 plants had none. The plants with pods averaged 14 pods/plant and 1.3 seeds/pod.



In the summer of 1978, the remainder of the seed from the 1977 plants was grown in the field ( $M_3$  generation) along with the seed from the greenhouse plants ( $M_4$  generation). At maturity all of these plants, 96 with 1977-78 greenhouse parents and 64 with the 1977 field parents, had a male-sterile phenotype. In addition, some of the plants from the  $M_3$  families and some from the  $M_4$  families had the multi-leaflet trait. However, the expression of this trait was not as pronounced as it was in the  $M_2$  generation and the segregation pattern of the trait did not suggest single gene inheritance.

All plants in the  $M_1$ ,  $M_2$ ,  $M_3$  and  $M_4$  generations had male-sterile phenotypes in the field environment which is good evidence that the trait is maternally inherited. Pods with seeds are rarely produced on genetic male-sterile ( $ms_1ms_1$ ) plants grown in the greenhouse, presumably due to a lack of insect pollen vectors. Therefore, the occurrence of full pods on 15 of 18  $M_3$  plants grown in the greenhouse may have been the result of an environmental restoration of male fertility. Environmental influences on fertility restoration of cytoplasmic male-sterility has been reported for other plant species, notably *Triticum* (Wilson, 1968) and *Nicotiana* (E. A. Wernsman, personal communication). Because the progeny of these plants were phenotypically male-sterile in the field, any male-fertility restoration which occurred in the greenhouse must have been nonheritable.

Study of these male-sterile lines is continuing in order to further characterize the phenotype, as to flower morphology and pollen viability. Methods of restoring fertility will also be investigated. A cytoplasmic male-sterile soybean line should be quite useful to soybean geneticists if a genotype can be found which will restore fertility in hybrid combination with the sterile line.

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1) The frequency of chlorophyll mutations in soybeans.

The first mutations of soybeans in the USSR were received by Leschenko A. in the 30's. In the late 50's, through direct selection of mutations, the first variety called 'Universal 1' was received in the USSR. The second strain--'Wonder of Georgia'--was received through crossing of induced mutants of soybeans.

Short season, high productive as well as resistant to cold and heat mutants were induced by many scientists. Application of mutagen factors for improving protein and oil content as well as a gap in negative correlations existing among economic traits is proved to be perspective.

This article gives the data on genetic activity of a number of chemicals and gamma rays in induction of chlorophyll mutations of soybeans.

Water solutions of the following mutagen factors were used in research: nitrosoethyl urea (NEU), nitrosomethyl urea (NMU), ethylenimine (EI), ethylenoxide (EO), nitrosodimethyl urea (NDMU) and dimethylsulphate (DMS). For induction of mutations, various gamma ray doses were used.

The research showed that soybeans have a wide spectrum of chlorophyll mutations that are revealed during the entire period of vegetation. The greatest number of such mutations were found within the period of plants' initial growth. The total frequency of chlorophyll alterations in investigated varieties that depends on the type of mutagen used is given in Table 1.

Such mutagens as NMU, NEU, EI and gamma rays induced high frequencies of alterations of that kind. Fewer mutations were given by EO, NDMU and DMS. In general, NMU induced the highest number of mutations with all strains. As to genetic activity, the investigated mutagen factors could be given in the following succession: NMU > NEU > EI > gamma-rays > EO > DMS > NDMU. The frequency of mutations given by one mutagen is not the same for different varieties (Table 2). The most mutable were varieties 'VNEEMK 9186' and 'Lanka'. 'Kirovogradskaya 4' and 'Peremoga' had average mutatability. 'Hybrid 89-10' had the lowest frequency of mutations. The mutagens that induced the higher frequency of mutations gave, as a rule, the wider spectrum (Table 3). Such mutagens as NEU, NMU, EI and gamma-rays in most cases had induced 2-3 types of chlorophyll mutations, while such low active mutagens as EO, NDMU and DMS gave only 1-2 types. Strain Peremoga, in comparison with other strains, had a wider range of mutation of such kind. In the second generation of varieties that were treated by chemical mutagens and gamma-rays, about 1,000 changed plants were singled out. Their selection significance and heredity are being studied at present. Among them there are strains with shortened growing season, high production, semi-dwarf, as well as mutants with changed content of protein and oil.

Table 1

Efficiency of chemical mutagens and gamma rays in induction of chlorophyll mutations dependable on strain's type genus

Type of mutagen	Lanka		Hybrid 89-10		Kirovograd-
	Investigated families	Frequency of mutations M <sub>2</sub>	Investigated families	Frequency of mutations M <sub>2</sub>	Investigated families M <sub>2</sub>
Control	154	0	95	1.05 $\pm$ 1.05	119
Gamma rays	367	3.27 $\pm$ 0.93	314	3.82 $\pm$ 1.08	303
NEU	149	8.72 $\pm$ 2.31	139	7.91 $\pm$ 2.29	295
NMU	230	10.87 $\pm$ 2.05	240	3.75 $\pm$ 1.26	380
EI	289	5.19 $\pm$ 1.30	259	3.47 $\pm$ 1.14	204
EO	372	1.07 $\pm$ 0.53	254	1.97 $\pm$ 0.87	339
NDMU	168	0.60 $\pm$ 0.60	291	2.74 $\pm$ 0.58	250
DMS	166	4.82 $\pm$ 1.66	223	0.90 $\pm$ 0.63	231

Table 1 (cont'd)

skaya 4	VNEEMK 9186		Peremoga	
Frequency of mutations	Investigated families M <sub>2</sub>	Frequency of mutations	Investigated families M <sub>2</sub>	Frequency of mutations
0	104	0	122	0.81 $\pm$ 0.71
3.30 $\pm$ 1.03	275	3.64 $\pm$ 1.13	373	4.29 $\pm$ 1.05
5.42 $\pm$ 1.32	206	10.68 $\pm$ 1.32	241	7.05 $\pm$ 1.65
11.05 $\pm$ 1.66	287	11.15 $\pm$ 1.86	302	9.93 $\pm$ 1.72
5.39 $\pm$ 1.58	258	10.85 $\pm$ 1.94	185	8.11 $\pm$ 2.01
1.77 $\pm$ 0.72	270	1.11 $\pm$ 0.64	364	3.02 $\pm$ 0.80
0.80 $\pm$ 0.56	247	0.40 $\pm$ 0.40	359	1.67 $\pm$ 0.68
0.43 $\pm$ 0.43	189	0	227	1.76 $\pm$ 0.87

Table 2  
The frequency of chlorophyll mutations  
depending on strain's type genus

Name of strain	Investigated families $M_2$	Families singled out with mutations pcs	Frequency of mutations
Lanka	1895	90	4.75 $\pm$ 0.49
Hybrid 89-10	1815	54	2.97 $\pm$ 0.40
Kirovogradskaya 4	2121	88	4.15 $\pm$ 0.43
VNEEMK 9186	1836	95	5.17 $\pm$ 0.52
Peremoga	2173	89	4.09 $\pm$ 0.42

Table 3  
Number of types of chlorophyll mutations induced  
with chemical mutagens and gamma rays

Mutagen influence	Number of types of chlorophyll mutations				
	Lanka	Hybrid 89-10	Kirovogradskaya 4	VNEEMK 9186	Peremoga
Control	0	1	0	0	0
Gamma rays 2.5	3	2	2	2	2
5.0	1	2	2	3	4
7.5	2	3	4	2	3
NEU 0.0125	2	3	3	4	1
0.025	2	4	2	1	-
0.05	-	-	1	-	4
NMU 0.00625	3	1	4	2	3
0.0125	2	2	2	2	4
0.025	2	2	3	2	3
EI 0.01	2	1	3	2	5
0.02	2	2	3	2	5
0.03	2	2	-	2	-
EO 0.05	1	2	3	-	3
0.10	1	1	1	1	3
0.20	1	1	1	2	1
NDMU 0.00625	-	1	-	0	2
0.0125	0	0	1	1	1
0.025	1	2	1	0	1
DMS 0.01	2	0	0	0	1
0.02	2	1	1	0	2



## 2) Morphological mutations of soybeans induced with chemical mutagens and gamma rays

The method of experimental mutagenesis is effective in investigating the hereditary alterations of plants with their further use in selection. Year by year the research in this field grows wider, covering new research institutions and crops.

The purpose of this article was to investigate the efficiency of a number of chemical mutagens and gamma rays in induction of morphological mutations in soybeans with their further use in selection of this crop.

The mutagen factors used gradually increased the frequency of visible morphological mutations. The frequency of mutations, in most cases, depended on the type of mutagen used, on its dose and variety (Table 1). The most effective among the mutagen factors turned out to be nitrosomethyl urea (NMU), nitrosoethyl urea (NEU) and gamma rays (Table 2). They induced 9.19-10.42% of visible mutations. Ethylenimine (EI) took the intermediate position. Ethylenoxide (EN), nitrosodimethyl urea (NDMU) and dimethylsulfate (DMS) had practically the same activity, having induced 4.26-4.63% of morphological mutations. The similar position the aforementioned mutagens had in frequency of chlorophyll mutations excepting gamma rays. In induction of morphological mutations, gamma rays appeared to be 3 times more effective than in induction of chlorophyll mutations. Genetic activity of this mutagen was not inferior to NEU and NMU but was higher in comparison with the other chemical mutagens. It shows that gamma rays are a highly active mutagen for soybeans and they should be used more widely in experimental mutagenesis research of this crop.

It was registered that the frequency of mutations had a rather complex dependence on the dose of mutagen (Table 1); e.g., NMU induced the highest output of mutations with 'Lanka' and 'VNEEMK 9186' at average dose, with 'Hybrid 89-10' at initial dose and with 'Kirovogradskaya 4' and 'Peremoga' at the definitive dose. Peremoga and Kirovogradskaya 4 gave high mutability; Lanka and VNEEMK 9186, average; Hybrid 89-10, low (Table 3). Approximately the same level was produced in chlorophyll mutations, excepting VNEEMK 9186. This variety took the first place as to the frequency of chlorophyll abnormalities but as to the number of morphological mutations it was less mutable. It is necessary to take into consideration that some mutagens can show high genetic activity only with definite genotypes; e.g., gamma rays induced the highest percentage of mutations with Hybrid 89-10, VNEEMK 9186 and Peremoga, at the same time they showed low efficiency with Lanka and Kirovogradskaya 4. It means that it is necessary to use several genotypes and mutagens for induction of a considerable number of hereditary changes. The used mutagen factors induced, in general, short season, long season, resistance to lodging, high production, tall, semidwarf, dwarf mutants with changed coloring of pubescence of stalk and pods, with changed number of pods on the plant. It is very important that short season mutants appeared rather often, because at present the problem of combination of short season and high productivity is one of the main problems in soybeans breeding in the USSR. That is why the use of induced mutations can bring considerable help in solving this problem. It was mentioned before that mutagens and varieties had a very similar succession according to their induction of morphological and chlorophyll mutations. However a more detailed analysis showed that this dependence has a complex character. Among the investigated varieties, for all mutagen treatments, only Kirovogradskaya 4 registered the high positive dependence between

Table 1  
Frequency of morphological mutations in different varieties of soybeans

Mutagen	Lanka		Hybrid 89-10		Kirovograd-
	Investigated families	Frequency of mutations	Investigated families	Frequency of mutations	Investigated families
Control	154	0.65 $\pm$ 0.65	95	1.06 $\pm$ 1.05	119
Gamma rays					
2.5	119	5.88 $\pm$ 2.16	132	7.58 $\pm$ 2.31	99
5.0	114	2.63 $\pm$ 1.50	97	9.28 $\pm$ 2.96	119
7.5	134	8.21 $\pm$ 2.38	85	3.53 $\pm$ 2.01	85
NEU					
0.0125	79	7.59 $\pm$ 3.00	72	4.17 $\pm$ 2.37	115
0.025	70	18.57 $\pm$ 4.68	67	5.97 $\pm$ 2.20	117
0.05	-	-	-	-	63
NMU					
0.00625	94	7.45 $\pm$ 2.72	93	4.30 $\pm$ 2.11	147
0.0125	68	17.65 $\pm$ 4.66	79	2.53 $\pm$ 1.78	155
0.025	68	8.82 $\pm$ 3.46	68	2.94 $\pm$ 2.06	78
EI					
0.01	99	6.06 $\pm$ 2.41	102	3.92 $\pm$ 1.93	93
0.02	90	5.56 $\pm$ 2.43	70	8.57 $\pm$ 3.37	111
0.03	100	4.00 $\pm$ 1.97	87	0	-
EO					
0.05	157	5.73 $\pm$ 1.86	72	0	120
0.10	118	4.24 $\pm$ 1.86	90	1.11 $\pm$ 1.11	120
0.20	97	6.18 $\pm$ 2.46	92	1.09 $\pm$ 1.09	99
NDMU					
0.00625	-	-	85	3.53 $\pm$ 2.01	-
0.0125	84	3.57 $\pm$ 2.04	74	4.05 $\pm$ 2.31	123
0.025	84	8.33 $\pm$ 3.03	132	1.52 $\pm$ 1.07	127
DMS					
0.01	73	4.11 $\pm$ 2.34	-	-	123
0.02	93	0	112	1.78 $\pm$ 1.26	108
0.04	-	-	93	0	94

Table 1 (cont'd)

skaya 4	VNEEMK 9186		Peremoga	
Frequency of mutations	Investigated families	Frequency of mutations	Investigated families	Frequency of mutations
0.84 $\pm$ 0.84	104	0.96 $\pm$ 0.96	122	4.09 $\pm$ 1.80
5.05 $\pm$ 2.21	88	6.81 $\pm$ 2.70	174	20.69 $\pm$ 3.03
4.20 $\pm$ 1.85	87	14.94 $\pm$ 3.84	114	14.04 $\pm$ 3.27
4.70 $\pm$ 2.31	100	11.00 $\pm$ 3.14	85	16.47 $\pm$ 4.05
13.91 $\pm$ 3.24	130	5.61 $\pm$ 2.03	153	13.07 $\pm$ 2.73
10.26 $\pm$ 2.82	76	7.89 $\pm$ 3.11	-	-
7.94 $\pm$ 3.43	-	-	88	5.68 $\pm$ 2.48
14.28 $\pm$ 2.90	99	7.07 $\pm$ 2.59	136	7.35 $\pm$ 2.24
18.71 $\pm$ 3.14	122	11.47 $\pm$ 2.90	84	7.14 $\pm$ 2.83
24.36 $\pm$ 4.89	66	4.54 $\pm$ 2.58	82	9.76 $\pm$ 3.29
10.75 $\pm$ 3.23	96	5.20 $\pm$ 2.28	93	10.75 $\pm$ 3.23
8.11 $\pm$ 2.60	102	3.92 $\pm$ 1.93	92	16.30 $\pm$ 3.87
-2	60	3.33 $\pm$ 2.34	-	-
5.83 $\pm$ 2.15	-	-	150	10.67 $\pm$ 2.53
5.00 $\pm$ 1.20	139	4.31 $\pm$ 1.73	94	6.38 $\pm$ 2.53
2.02 $\pm$ 1.42	131	2.29 $\pm$ 1.31	120	5.00 $\pm$ 2.00
-	78	1.43 $\pm$ 1.35	128	7.03 $\pm$ 2.27
1.63 $\pm$ 1.15	78	5.13 $\pm$ 2.51	136	3.68 $\pm$ 1.62
3.94 $\pm$ 1.73	91	5.49 $\pm$ 2.40	95	8.42 $\pm$ 2.86
4.88 $\pm$ 1.94	101	0.99 $\pm$ 0.99	108	6.48 $\pm$ 2.38
4.63 $\pm$ 2.03	88	10.23 $\pm$ 3.25	119	7.56 $\pm$ 2.43
5.32 $\pm$ 2.33	-	-	94	6.38 $\pm$ 2.53

the frequency of chlorophyll and morphological mutations (Table 4). The selection value of received mutant strains is being investigated at present.

Table 2  
Influence of type of mutagen on the frequency  
of morphological mutations in soybeans

Mutagen factor	Number of families	Frequency of mutations, %
Gamma rays	1632	9.19 $\pm$ 0.72
NEU	1030	9.32 $\pm$ 0.90
NMU	1439	10.42 $\pm$ 0.81
EI	1195	6.28 $\pm$ 0.70
EO	1599	4.63 $\pm$ 0.52
NDMU	1315	4.26 $\pm$ 0.56
DMS	1206	4.39 $\pm$ 0.59

Table 3  
Frequency of morphological mutations  
in different varieties of soybeans

Name of variety	Investigated families	Frequency of mutations, %
Lanka	1741	7.47 $\pm$ 0.63
Hybrid 89-10	1702	3.47 $\pm$ 0.44
Kirovogradskaya 4	2096	8.25 $\pm$ 0.60
VNEEMK 9186	1732	6.06 $\pm$ 0.57
Peremoga	2145	9.51 $\pm$ 0.63

Table 4

Coefficient of correlation between the frequency of  
morphological and chlorophyll mutations in soybeans  
depending on the type of variety

Name of variety	Coefficient of correlation
Lanka	+ 0.21
Hybrid 89-10	- 0.02
Kirovogradskaya 4	+ 0.96
VNEEMK 9186	+ 0.11
Peremoga	- 0.17

V. I. Sichkar



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#### ADDENDUM

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